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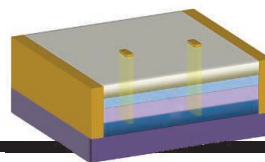
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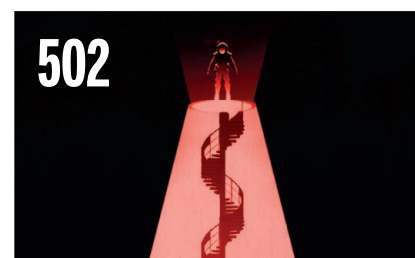
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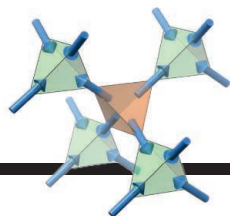
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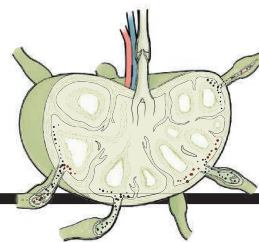


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False-color microscopic view of a reduced graphene oxide electrode (black, center), which hosts the large (on the order of 20 micrometers) lithium hydroxide particles (pink)

that form when a lithium-oxygen battery discharges. Lithium iodide aids in the removal of the particles upon charging, resulting in an efficient and highly energy-dense battery with potential applications in portable electronics, transportation, and grid storage. See page 530.

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Zero tolerance. Period.

Earlier this month, famed astronomer Geoff Marcy's sexual harassment of female students was exposed. He has since resigned from the University of California, Berkeley, in the face of concerted pressure from peers and students. It is unconscionable for someone to use academic power to be a sexual predator, but the reality is that Marcy operated in an academic culture that turned a blind eye to such behavior.

In academic life, power comes from the perception that a person is more influential than that individual's peer group. This leads to invitations to give talks and to serve on panels and editorial boards—activities that further increase a person's influence. Such imbalances in influence are stark in the sciences, where students and postdoctoral fellows train under established professors as part of their career path. That a trainee's future is in the hands of an advisor is an important source of the power imbalance that provides opportunities for abuse. Recent research confirms that most sexual misconduct is perpetrated by men who outrank the women they prey on,* and science is a discipline where the faculty balance is tilted; most senior professors are male.

In my own field of anthropology, I have been aware of the problem of sexual misconduct, and I understand just how pervasive, insidious, and devastating this behavior is. Too many young women have been discouraged, and too many careers have been blighted, to just stand by and do nothing about a culture that fails to identify and call to account male colleagues who have behaved inappropriately. What can those like me, a senior tenured male professor—an “alpha male” in the academic world—do to change this?

Any professor who fails to recognize that exploiting academic seniority to solicit sexual favors is reprehensible has no place in academia. Senior male professors must make it clear that there are no “gray areas” and

lead efforts to ensure an ongoing zero tolerance policy in their departments.

Male professors have a special responsibility to be strong allies of the women affected by sexual misconduct. Many women who have been the target of inappropriate behavior, or have even heard of such incidents, believe that their careers will be jeopardized if they speak out. To work toward something for years, only

to have it derailed by an unscrupulous superior or by malicious rumor, is a frightening prospect. Equal academic opportunity will not exist as long as individuals have to adjust their careers to avoid exposure to sexual predation. Women must be supported by their male colleagues, especially those with the greatest influence, when they speak out, make formal complaints, or press criminal charges. We should not wait for traumatized junior colleagues to demonstrate the greatest courage before those with the greatest power show any.

At the very least, any scientist should think twice before collaborating with those who use their research reputation to harass female colleagues, and before inviting them to conferences.

Why? Because every paper they publish, talk they give, and conference they attend enhances the influence they have abused. If perpetrators are made to pay a professional cost, their influence will wane, depriving them of further opportunities to prey on women. More importantly, male faculty must report concerns to institutional authorities. The more frequently a department head or a dean learns of concerns, the more likely it is that behaviors will be recognized as a pattern of misconduct.

Sexual harassment in the sciences occurs in many circumstances and settings, but the silence of the past must be replaced by action. The untenured are brave to speak out, but powerful male voices must join in to make sure we level this particular playing field. Alpha males are the problem. Alpha males need to be part of the solution.

– Bernard Wood



“It is unconscionable for someone to use academic power to be a sexual predator...”



Bernard Wood is the University Professor of Human Origins at the Center for the Advanced Study of Human Paleobiology, George Washington University, Washington, DC. E-mail: bernardawood@gmail.com

“They say every piece of bacon takes 8 minutes off your life. I should have died in like 1752.”

Tweet by @Pillownaut, aka NASA contractor **Heather Archuletta**, reacting to a World Health Organization finding that processed meat causes cancer.

IN BRIEF

A sweeping panorama of the Milky Way



A small section of the Milky Way photo, showing the volatile Eta Carinae star system.

Astronomers at Germany's Ruhr University Bochum have unveiled a 46-billion-pixel panorama of the Milky Way, the largest such image ever assembled. The stunner was stitched together from 268 smaller images taken from an observatory in northern Chile between September 2010 and May 2015. Some of the areas (such as the one pictured) were observed as many as 272 times, because astronomers were surveying the skies for stars or other objects whose brightness varied over time. The team identified more than 64,000 variable stars, nearly 90% of which were previously unknown, the researchers reported online last week in *Astronomical Notes*. The team's 194-gigabyte image covers a 1323-square-degree swath of the sky, an area more than 6500 times the size of the full moon. At <http://astro.vm.rub.de/> you can view the entire ribbon of the Milky Way or zoom in on your favorite star or nebula to see it in several different wavelengths.

AROUND THE WORLD

Government shakes up science

CANBERRA | Australian Prime Minister Malcolm Turnbull, who replaced Tony Abbott on 15 September, this week appointed neuroscientist Alan Finkel to be the country's chief scientist. Finkel, who says that two of his priorities will be to boost the nation's innovation record and to set it on the road to a fossil-free future, will take up the post in January. The government also announced last week that it will not help establish an Australian Consensus Centre (ACC), a version of climate contrarian Bjørn Lomborg's Copenhagen Consensus Center. The government of former Prime Minister Tony Abbott, who in 2009 dismissed climate change as “absolute crap,” intended to support an ACC that would study overseas aid, Australian prosperity, agriculture, and regional issues (*Science*, 24 April, p. 377). Abbott's government had failed to appoint a science minister for over a year. <http://scim.ag/ACCscrapped>

Scientists study giant hurricane

CUIXMALA, MEXICO | Hurricane Patricia, the strongest hurricane on record, made landfall on the west coast of Mexico on 23 October. Fueled by an unusually deep layer of warm water in the eastern Pacific—an effect of El Niño—Patricia grew from a Category 1 to a Category 5 storm in just 1 day, with winds topping 200 miles per hour (322 km) as it neared land. Patricia came ashore along a sparsely populated stretch of coast, causing few casualties. Patricia was one of the first



Patricia, seen from the International Space Station.

hurricanes to be studied by the WB-57, a U.S. Navy bomber outfitted by NASA to fly above hurricanes and study their interaction with the stratosphere. “We’ve never been able to measure the upper third or so of a hurricane,” says Kerry Emanuel, a meteorologist at the Massachusetts Institute of Technology in Cambridge. “I think we’re going to learn a lot from that.” <http://scim.ag/Patriciainitor>

Malaria vaccine needs pilot tests

GENEVA, SWITZERLAND | The world’s first malaria vaccine, known as RTS,S or Mosquirix, needs to be tested more before any decision can be made on its wider use, according to the World Health Organization’s Strategic Advisory Group of Experts on Immunization (SAGE). RTS,S, developed by GlaxoSmithKline, is not a great vaccine; it protects only about a third of young children against severe malaria (*Science*, 1 May, p. 481). Children need four shots of RTS,S, three given a month apart and the fourth 18 months later—a tough schedule to follow in malaria-affected countries. The panel recommended three to five large pilot projects enrolling up to 1 million children total to determine how to deliver the vaccine effectively. Plans for the studies could be ready by the time SAGE meets again in April next year. <http://scim.ag/malariaSAGE>

Budget plan could help research

WASHINGTON, D.C. | The U.S. Congress is moving toward adopting a belated budget agreement that would give domestic science agencies a bit more money in 2016. The tentative agreement would add \$50 billion to a current \$1.016 trillion limit on discretionary spending, with the increase divided equally between military and civilian agencies. It’s not as large as the \$71 billion boost requested by President Barack Obama, and legislators must still agree on how to offset the additional spending. But the deal will allow congressional spending panels to bump up allocations to individual agencies for the fiscal year that began on 1 October. The new agreement also adds \$30 billion to FY 2017 spending levels. But it won’t permanently remove a 10-year spending cap imposed by a 2011 budget law that led to the dreaded “sequestration” cuts in 2013.

Better detection ups TB numbers

GENEVA, SWITZERLAND | The number of people who developed active cases of tuberculosis (TB) jumped from an estimated 9 million in 2013 to 9.6 million in



A wetland outside Hong Kong, China.

China’s coastal wetlands nearing critical red line

Along China’s coastline, rapid development has transformed marshes and mudflats into ports and urban sprawl. The decline of wetlands is nearing a “red line,” a critical threshold below which the losses could inflict severe and lasting harm on ecosystems—driving numerous migratory bird species to the brink of extinction and jeopardizing nearly 20% of the world’s fisheries, a new report from Chinese and U.S. scientists warns. Half of China’s coastal wetlands have disappeared over the past 50 years, as well as 70% of its mangrove forests and 80% of near-shore coral reefs, according to the analysis, released last week by China’s State Forestry Administration, the Chinese Academy of Sciences’ Institute of Geographic Sciences and Natural Resources Research, and the Paulson Institute, a nonprofit based in Chicago, Illinois. On 25 April, China’s central government decreed that no fewer than 53.33 million hectares of wetlands must be conserved. However, the new report forecasts that if current and planned coastal reclamation continue unabated, by 2020 the government’s red line “will be broken.” <http://scim.ag/chinawetlands>

2014, according to a new global report by the World Health Organization (WHO). WHO attributes the spike to improved data, particularly from Indonesia, which doubled its estimated caseload from the previous year to 1 million. “The trend in TB incidence globally as well as in Indonesia is still downward since around 2000,” the report stresses. Countries are supposed to report everyone who develops symptomatic forms of the disease, but only

reported 6 million new cases last year; the other 3.6 million in the WHO estimate were either undiagnosed or unreported. WHO estimates that TB killed 1.5 million people in 2014, the same number as in 2013; more than 25% of these deaths occurred in people who were infected with HIV, and 8% had multidrug-resistant TB. For the first time in decades, there were more deaths from TB than from HIV/AIDS, which killed 1.2 million people last year.



At Oxia Planum, shown here in an image from NASA's Mars Reconnaissance Orbiter, clay-rich rock might hold clues left by ancient organisms.

PLANETARY SCIENCE

Europe's Mars rover to target ancient wetland

Deep-drilling ExoMars mission will probe long-buried clay formation for signs of life

By **Daniel Clery**

European planetary scientists are still building the roving laboratory they plan to send to Mars in 2018, but now they know where it will land: Oxia Planum. Clay deposits and landforms suggest this region once hosted lakes,

rivers, and a delta, making it just the sort of place where you might dig into ancient soil to find signs of life, if any has ever existed on the Red Planet. And that is the mission of the ExoMars 2018 rover, the first dedicated search for martian life since the Viking landers of the 1970s.

The landing site was chosen on 21 October after 2 days of intense discussions at the European Space Agency's (ESA's) technology center in Noordwijk, the Netherlands. It beat out three other candidates. "It was difficult," says ExoMars project scientist Jorge Vago. "All four sites are very good. There were no dogs there." The four site teams had spent 18 months preparing for the meeting; some members had previously proposed the same sites for NASA's Curiosity rover, which is currently exploring Mars. "That's almost 10 years' work," Vago says. "It was emotionally tough for the four teams."

But researchers seem happy with their decision. "This site guarantees most of what we want in terms of science and engineering," says one member of the ExoMars landing site selection working group, astrophysicist Jean-Pierre Bibring of the Institute of Space Astrophysics in Orsay, France.

ExoMars is a multipart mission involv-

ing ESA and its Russian counterpart, Roscosmos. First to launch, in 2016, will be the ExoMars Trace Gas Orbiter (TGO) plus a stationary lander, called Schiaparelli. TGO will survey the martian atmosphere for traces of methane and other gases that could indicate biological activity, whereas the lander will test entry and descent technology along with the landing system, which relies on parachutes and thrusters.

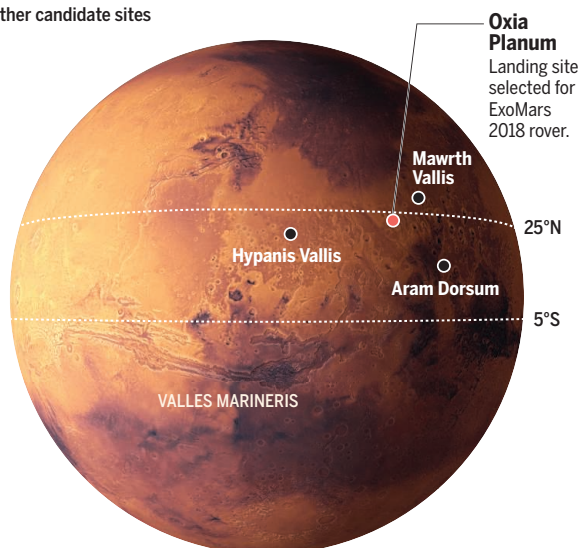
Two years later the rover will lift off for its 9-month journey to Mars. After the descent module lands at Oxia Planum and releases the rover, it will become an environmental monitoring station. The rover will set off to explore nearby terrain, carrying nine instruments for mapping the landscape, studying minerals and geology, and analyzing samples. It will also wield a drill that can penetrate 2 meters below the surface, analyzing the rocks it probes with a multispectral imager in its tip and extracting samples for further analysis in the rover's onboard laboratory, which will be equipped to identify organic molecules.

In picking a site to explore, researchers drew heavily on data from spacecraft orbiting the planet, including NASA's Mars Reconnaissance Orbiter (MRO) and ESA's Mars Express. One

A site fit for life

The choice of landing site for the ExoMars rover had to balance the greatest chance of finding signs of life with the engineering constraints of landing safely on a hostile planet.

● Other candidate sites



“must” for all four ExoMars sites was clay. On Earth, clay is deposited by water and is excellent at preserving the remains of ancient organisms. The near-infrared sensors on both MRO and Mars Express discovered a decade ago that much of Mars is covered with clay formations hundreds of meters thick, Bibring says—strong evidence that water must have pooled on the surface for long periods in the past.

Researchers also wanted a site where the clays were covered by other material for billions of years and then recently uncovered by wind erosion. Because ionizing radiation from space easily penetrates Mars’s thin atmosphere and probably would destroy any organic molecules near the surface, long-buried clay is more likely to harbor biomarkers today.

Both Oxia Planum and another site, Mawrth Vallis, tick both boxes. Oxia Planum won out because its landing trajectory is better in 2018. The site is a shallow basin with layered deposits of iron and magnesium phyllosilicate clay. For most of the past 3.6 billion years, the clay has been covered by a layer of dark, possibly volcanic, material, which has eroded away in the past 100 million years. Bibring hopes the rover’s data will let him study clay from different layers to learn how its composition changed over time. “If we can find biomarkers, they would indicate which environments favored and preserved the emergence of life. That’s fundamental,” he says, and might help explain the origins of life on both Earth and Mars.

Oxia Planum offers another attraction: On its east side sprawls a 15-kilometer-wide, fan-shaped feature that researchers think could be an ancient river delta or alluvial fan. If so, it could give the rover a second opportunity to look for life, because it could indicate that water flowed on Mars again long after the inundation that deposited the clay. “Maybe that [later period] might have concentrated and preserved things,” Bibring says.

ExoMars has had a long and often difficult history. It started out as a standalone ESA rover in 2005, but as ambitions grew it became a multipart mission in collaboration with NASA in 2009. NASA pulled out because of budget conflicts in 2012, and the mission ended up as a partnership with Roscosmos. While ESA negotiates with companies over building the 2018 spacecraft, it is testing models on the ground—and is running behind schedule. “You want a certain amount of margin, and right now it is very thin to nonexistent,” says Vago, who is working to adjust the schedule. The project does have the fallback option of delaying launch of the rover until 2020; if that proves necessary, Mawrth Vallis could come back into the running. ■

NEUROSCIENCE

Lifelong memories may reside in nets around brain cells

Studies suggest key role for perineuronal networks of proteins and sugars in long-term memory

By Emily Underwood, in Chicago, Illinois

In 1898, Italian biologist Camillo Golgi saw something odd in the slices of brain tissue he examined under his microscope: weblike lattices surrounding many neurons. Golgi could not discern their purpose, and many dismissed the nets as an artifact of his staining technique. For the next century, the lattices remained largely obscure. But last week at the annual meeting of the Society for Neuroscience here, researchers offered tantalizing new evidence that holes in these nets could be the storage sites for long-term memories.

Perineuronal nets (PNNs), as they are known today, are scaffolds of linked proteins and sugars that resemble cartilage. A growing body of research suggests that PNNs may control the formation and function of synapses, the microscopic junctions between neurons that allow cells to communicate and that may play a role in learning and memory, says neuroscientist Sakina Palida, a graduate student at the University of California, San Diego. At the meeting, Palida and colleagues attracted a crowd with several new findings that support the hypothesis.

Just how memories are stored in the brain—particularly long-term ones—is one of the most pressing questions in neuroscience. Most of the proteins inside neurons are constantly being replaced—anywhere from every couple of days to every few hours—so it’s not clear how the

cells can retain information for decades. Some scientists believe that lifelong memories must somehow be encoded in a more persistent, stable molecular structure.

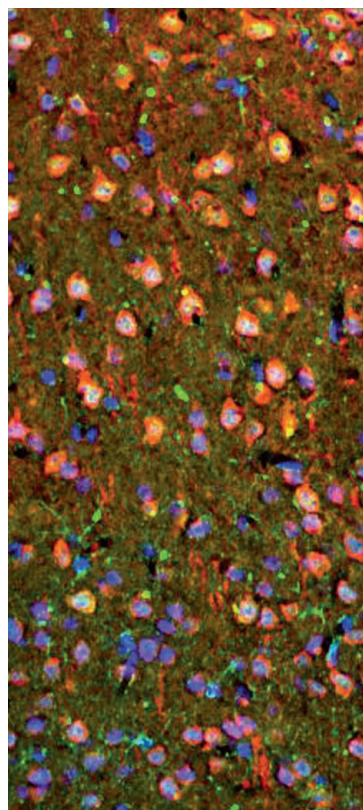
Palida’s adviser, Nobel Prize-winning chemist Roger Tsien, recently began to explore whether PNNs could play that role. He was inspired in part by evidence that

destroying the nets in some brain regions can erase fearful memories, as well as reverse ocular dominance, the brain’s tendency to prefer input from one eye over the other, which develops early during maturation (*Science*, 4 September 2009, p. 1258). Another clue came from recent studies linking abnormal PNNs to brain disorders, including schizophrenia and a form of intellectual disability named Costello syndrome.

Tsien’s group started by testing whether PNNs are durable, as a memory substrate must be. The team confirmed that proteins contained in the PNNs can survive for at least 180 days—almost a lifetime for a mouse. With the help of a new fluorescent labeling technique developed

by Palida, they also found PNNs throughout the brain, showing that they are not limited to just a few brain areas, as some studies had suggested.

Next, Palida and colleagues explored how PNNs interact with synapses, which are thought to form and grow stronger as memories are created and reinforced, and weaken or even disappear as we forget. After growing neurons in a petri dish and allowing the PNNs surrounding



Perineuronal nets (green) surround neurons (red) in the mouse brain.

them to develop, Palida treated the cells with BDNF, a brain chemical known to stimulate neurons to form new connections. Electron microscopy showed that wherever new synapses had emerged, the PNN's tight-knit lattice had developed holes, as if to accommodate the new connections.

In a separate experiment, the group found that genetically engineered mice lacking an enzyme that normally degrades the PNN performed badly on a learning task in which they had to associate a shock with a beep—a sign that they had trouble forming new memories. Taken together, the new data suggest that holes in the PNN provide openings for new synapses to form, as well as structural support that sustains those connections and helps memories endure, Palida says.

The results are “pretty cool,” and fit with growing evidence that loose or degraded PNNs increase neural plasticity, says Serena Dudek, a neurobiologist at the National Institute of Environmental Health Sciences in Research Triangle Park, North Carolina. At the meeting, her own lab presented evidence supporting the hypothesis that PNNs form during so-called critical periods: set windows of time in which functions such as vision develop.

Little is known about what builds the nets, although some evidence suggests that neurons and brain cells called glia both play a role. Barbara Sorg, a neuroscientist at Washington State University, Vancouver, notes that environmental factors can also loosen up or tighten PNNs. Her research has shown that cocaine addiction, for example, seems to lead to the production of more PNNs in animals, suggesting a potential mechanism for the intense and persistent memories that form as a result of drug abuse, she says.

At the meeting, Sorg's group presented evidence that destroying the PNN in some regions can erase drug-associated memories, suggesting that the process may be reversible. Don't look for PNN-dissolving therapies anytime soon, however: The enzyme used to break down the PNN in animals is a “very blunt tool,” Sorg says, and there's no knowing how it would affect a person.

To determine PNNs' true role in memory, scientists will need a way to watch how they change over time in living animals, says Takao Hensch, a molecular biologist at Harvard University. To do that, Tsien's group plans to create transgenic rodents with fluorescent PNNs, so that their development can be traced as the animal ages. So far, Palida says, PNNs appear to be an “ideal substrate for very long-term maintenance of memory over time.” ■

PALEONTOLOGY

How some of the world's biggest dinosaurs got that way

Fresh data reveal titanosaur growth stages, from egg to adult

By Michael Balter in Dallas, Texas

When all dinosaurs except the birds went extinct 66 million years ago, they exited in grand style: The last dinos included the largest creatures ever to walk the earth. These were the titanosaurs, a group of long-necked sauropods including *Argentinosaurus*, a South American species stretching nearly 40 meters long and weighing more than 70 tons—as much as 15 adult elephants and more than twice as much as the classic sauropod, *Apatosaurus*.

Paleontologists and the public alike have been fascinated by these beasts, which “represent a possible upper ceiling for how large life can get on land,” says Philip Mannion, a paleontologist at Imperial College London. Yet the titanosaur fossil record has been pretty scrappy—just three complete skulls have been found—leaving major mysteries about these behemoths. Researchers still “need to learn a lot more” about how and why these animals grew so big, and how they managed to move their massive bodies, says Luis Chiappe, director of the Dinosaur Institute at the Natural History Museum of Los Angeles County in California.

The picture is beginning to fill in, however. At a special session at the annual meeting of the Society of Vertebrate Paleontology

here, researchers presented new fossils that chart titanosaur growth from embryo to adult, and help explain how they moved.

Perhaps the most spectacular addition to the record was a titanosaur egg that apparently was smuggled out of Argentina years ago, but was recently donated to a museum and restored to science. (Collector Terry Manning, who arranged the donation, was in the audience and won a round of applause.) To image the egg's interior, a team led by paleobiologist Martin Kundrát of Uppsala University in Sweden probed with the high-energy x-ray beam at the European Synchrotron Radiation Facility in Grenoble, France.

The scan provided the most detailed look yet at a sauropod embryo's skull, Kundrát said. It revealed that the facial bones ossified first, whereas the jaw and other skull bones were still soft tissue. Although eggs of many other dinosaurs are common, only one clutch of titanosaur eggs has been found, all from a single site in Patagonia. The new embryonic skull looks like a different species, Kundrát said, adding one more taxon to the slim file on titanosaur embryos.

Another talk shed light on how titanosaurs developed after birth. Paleontologist Kristina Curry Rogers of Macalester College in St. Paul unveiled one of the smallest hatchlings ever discovered: a tiny titanosaur from Madagascar, *Rapetosaurus krausei*, com-



The titanosaur *Argentinosaurus* was one of the largest land animals ever.

PHOTO: © TOM WAGNER/ALAMY STOCK PHOTO

plete with parts of the fore- and hindlimbs, pelvis, and vertebral column.

Adult *Rapetosaurus* were moderately sized, about 3 meters tall at the hip and 15 meters long. The baby was only about 35 centimeters tall at the hip, yet it looked like a miniature adult, Curry Rogers told the meeting. The bones also showed signs of early and rapid growth and remodeling, suggesting that *Rapetosaurus* grew fast and relied little on parental care even from a young age.

Earlier sauropods such as *Diplodocus* had a distinctive juvenile stage and grew more slowly; rapid growth “seems to be a titanosaur specialty,” says paleontologist Martin Sander of the University of Bonn in Germany. Paleontologist Michael Habib of the University of Southern California in Los Angeles says a baby *Rapetosaurus*, unencumbered by an adult’s size and weight, “might have been able to gallop and turn rapidly, even though the adults could not.”

Indeed, the necks and tails of many adult titanosaurs would have been even more massive, and hard to control, than those of other sauropods. The latest finds from a dig in New Mexico that Habib and Chiappe are running suggest one solution to the biomechanical challenge. The researchers unearthed an extended segment of a titanosaur neck that has nine long, thin cervical ribs, structures that appear to be ossified tendons and run in parallel to the vertebrae.

The ribs are 1.8 meters long, spanning three vertebrae each. Habib wondered whether these structures might have acted as springs to help stabilize the long neck as the titanosaur moved about. He plugged the dimensions of the fossils into a mathematical model for spring action, and found that the cervical ribs would indeed dampen sideways or up-and-down oscillations, especially if the neck were elevated. (Recent biomechanical modeling suggests sauropods could raise their necks to feed as giraffes do.)

Habib also modeled the action of the neck muscles, which could have helped the animal cope with the challenge of pumping blood to its head, many meters above: As the neck moved, the pressure of muscles against the neck arteries would have pushed the blood up, reducing by 25% the size of the heart needed to pump to the brain.

“I like this hypothesis,” Sander says. “There is good evidence that some sauropods elevated their necks somewhat, but we had the cardiovascular problem that the heart would not be strong enough to pump the blood into the head.” This helps resolve that issue, though Sander notes that the “pumping method would not work when the animal is not moving.” Even so, the new research suggests that titanosaurs marched to extinction with heads held high. ■



Researchers plan to chart New Yorkers’ activities, including any skate sessions in Manhattan’s Central Park.

BIG DATA

Proposed study would closely track 10,000 New Yorkers

Twenty-year household surveillance project aims to gather data first, produce hypotheses later

By Kelly Servick

In a year and a half, 2500 households in New York City may receive a startling request: to allow a team of scientists to monitor in intimate detail how they lead their lives over the course of 20 years—where they go, what they eat, who they talk to, what they buy, and how their bodies grow, change, and deteriorate. That’s the ambition of the Kavli Human Understanding through Measurement and Analysis (HUMAN) Project, a planned effort to amass vast amounts of data about health, behavior, and lifestyle for social science and biomedical researchers to mine. “It’s a really extraordinary project,” says Eric Topol, a physician and geneticist at Scripps Research Institute in San Diego, California, who is not involved in the effort.

Earlier this month, halfway through 3 years of planning, the HUMAN project released a preliminary study design that includes a mind-boggling wish list of measurements. All 10,000 participants would submit blood, saliva, hair, and stool samples every 3 years to reveal their genet-

ics, chemical exposure, and microbiome composition. They would provide access to medical records, educational records, and financial documents. Their smartphones would constantly log their location, activity, and social interactions, and would invite them to take weekly questionnaires.

“We’re frustrated as natural scientists and social scientists by the lack of data that’s really available at that fine granularity,” says project director Paul Glimcher of New York University (NYU) in New York City, who describes himself as a neuroscientist, psychologist, and economist.

The unprecedented data trove, he says, would allow researchers to look for unexpected correlations, in contrast to smaller scale health or behavioral studies that set out to test a single hypothesis or answer a set of research questions. The new project’s approach is a sign of a broader “generational shift,” suggests computer scientist Larry Smarr of the University of California, San Diego. “In a data-poor world, you have to come up with a topic you can focus enough to get a little bit of data,” he says, “but if you’re in a data-rich world, the data

generates its own hypotheses, which then have to be verified.”

To make the benefits of the HUMAN Project more concrete, its leaders have drawn up a list of 25 “grand challenges” for the study, laid out in white papers written by outside experts. The first four were published in last month’s issue of the journal *Big Data*. One challenge is to tease out connections between lifestyle and dementia, a major public health burden. Smaller studies have shown that such factors as exercise and social activity can help stave off mental decline, but which variables are most important? Another challenge would correlate tobacco purchases with epigenetic changes in tissue to assess how smoking habits influence molecular events that can lead to cancer or other diseases.

Backers say part of the strength of the project is its location in New York City: The five boroughs offer a well-developed infrastructure, which provides records of electricity use, garbage volumes, and noise complaints. Geographic information systems can track weather patterns and environmental toxins down to the resolution of a city block. And the city’s population provides plenty of ethnic variety. To get privacy-loving New Yorkers to agree to 20 years of surveillance, the project would offer money, access to personal and population-level data, and “items and activities that build community good will,” such as birthday cards, branded merchandise, or opportunities to participate in a public forum, according to the published plans.

The planning phase is supported by NYU and the philanthropic Kavli Foundation. Project leaders haven’t yet released a total budget; they’re still seeking partners to help fund a 2017 launch and soliciting input from scientists at meetings, such as at the annual meeting of the Economic Science Association last week in Dallas, Texas.

The HUMAN Project is not the only big-data health effort underway—Topol cites geneticist J. Craig Venter’s Human Longevity Inc., which plans to collect 100,000 genomes a year plus an array of other biological data. But no project is “as comprehensive, as longitudinal, and really focused on both behavior and environment,” Topol says.

Still, he expects some pushback. “Some pure scientists will say, ‘How can you spend all this money if you don’t really have a discrete hypothesis?’”

The project’s leaders are walking a line between the two camps. “Discovery science is controversial ... I was raised as a hypothesis-driven scientist, so I understand that,” Glimcher says. “It’s incumbent on anybody who’s trying to run a large study like this to make sure there are guaranteed payoffs.” ■

POLAR SCIENCE

Mysterious Antarctic lake will remain out of reach

Cash-strapped Russian expedition curtails drilling plans as it sets out for the southern continent

By Carolyn Gramling

Budget cuts and a weak ruble have forced Russia to put on ice one of its highest profile science projects: a 20-year odyssey to drill into a lake under the Antarctic Ice Sheet in search of long-buried life. Postponing the pricey effort to penetrate Lake Vostok is a sign of hard times in Russia’s broader Antarctic program. Some outside scientists, meanwhile, think it is time to rethink the complex project.

In 2012, the Russian Antarctic Expedition (RAE) completed drilling through nearly 4 kilometers of ice to reach the surface of Vostok, the continent’s largest subglacial lake. The lake has been isolated for 15 million years, and scientists speculate that its stygian waters might contain unknown life forms—living clues to the sorts of organisms that might exist elsewhere in our solar system. The team collected its first samples from the lake in February 2012. In March 2013, RAE touted the discovery of an unclassified microbe, but others worried that the samples were contaminated by microbes in the kerosene used as a drilling lubricant. Analyses of samples collected this past January, which the Russian team asserts are pure lake water, have not yet been released.

RAE intended to penetrate the lake again during the coming austral summer, until Russia’s austerity budget nixed that plan. The government has slashed RAE’s budget by about 10%, reducing it to about \$16 million. But given the ruble’s fall, that’s an effective cut of about 35%, as many of the services needed by the Antarctic program—plane tickets to the staging ground in southern Chile, for example—are purchased with foreign currency. Rumors started circulating that Russia would suspend its Antarctic program altogether.

That did not come to pass. “Gossips about [RAE’s] death are obviously exaggerated,” says Valery Lukin, head of RAE.

On 19 October, the Russian Academy of Sciences Scientific Council, which oversees RAE’s research, agreed to keep all five Russian Antarctic bases open year-round. The upcoming expedition will include 592 people; research staff were slated to depart for Antarctica from St. Petersburg on 29 October aboard the research vessel *Akademik Fyodorov*. But the program will be hobbled: The number of projects that won council backing fell to 56, from 70 last year. And RAE climatologist Alexey Ekaykin notes that Vostok station is “completely obsolete” and long overdue for renovation.

Some experts hope Russia will use the downtime to revise its drilling plans. Under pressure from the scientific community, RAE halted drilling at Vostok from 1998 to 2006

while it devised a plan that would avoid contaminating the lake. But some scientists contend that the drilling could still introduce contaminants. “This [hiatus] will give them time to rethink their science and drilling strategies,” says John Priscu, a microbiologist at Montana State University,

Bozeman. He would like to see RAE close its kerosene-filled borehole and start fresh with a “clean access strategy” such as hot-water drilling, a technique Priscu and others used to access another Antarctic subglacial lake in 2013. The stakes for maintaining Vostok’s purity are high, he says. The lake is “a prime analog site in our search for life on other icy worlds in our solar system.”

David Pearce, a microbiologist at the British Antarctic Survey and the University of Northumbria in Newcastle upon Tyne, U.K., agrees that “it might be prudent to hold off for a year.” But he hopes the Russians’ setback is temporary. He was part of a U.K. team that in December 2012 planned to use hot-water drilling to penetrate a third subglacial lake but ran afoul of technical problems. “We really don’t want to see each other failing,” Pearce says. “We need the momentum to get funding to try and go back and do it again.” ■

With reporting by Vladimir Pokrovsky.



WHO Director-General Margaret Chan says her job has become "more and more political and more and more intense."

Q&A

Crisis manager with 194 bosses

WHO Director-General Margaret Chan wants member countries to put their money where their mouths are

By Kai Kupferschmidt, in Berlin

As director-general of the World Health Organization (WHO), Margaret Chan is often ranked among the most powerful women in the world. But her agency appeared to be powerless to stop a devastating epidemic of Ebola last year. Critics have slammed WHO's performance, and reviews have called for drastic reforms (*Science*, 17 July, p. 223).

Chan is used to crises; as director of health in Hong Kong, China, she fought devastating outbreaks of bird flu and SARS before taking WHO's top job in 2006. *Science* talked to Chan on 10 October here, where she spoke about the lessons from Ebola and the dangers of antimicrobial resistance at a meeting of the G7 health ministers. Here are excerpts of the interview, edited for brevity and clarity; a longer version is at http://scim.ag/Chan_Ebola.

Q: In retrospect, it seemed obvious that the Ebola outbreak would become huge. A deadly virus in a part of the world that had no experience with it, health systems neglected during years of civil war, and a population that was highly mobile across borders, distrustful of governments, and more inclined to seek out traditional healers. Why did WHO miss the significance of all of this?

A: With the benefit of hindsight, the mistrust is a major problem. ... Instead of sending patients to a treatment center as early as possible, people in the community kept their loved ones at home and nursed them. It was

like a peat fire spreading underground. ... Information was not flowing up. That is a big problem. You cannot manage what you don't see and what you don't know. I think all of us underestimated the context of these countries. An old disease in a new context gave us surprises.

Q: WHO's Emergency Committee for Ebola recently criticized 34 countries for imposing "disproportionate" restrictions on travel to and from the Ebola region; it said this is hampering the response and recovery efforts.

A: This is a big problem. Countries that are affected by an outbreak should be transparent and report their diseases. Countries that are not directly affected should not impose trade or travel measures over and above what is recommended by WHO. This is part of the International Health Regulations [IHR], an international treaty with the good intention of building a collective defense system against a common threat. But the implementation of the IHR is very poor; there is a lot of disincentive. Why should I report? The minute I report, you impose a trade ban and travel ban on me. That is why we need to review the IHR and change them to provide incentive instead of disincentive.

Q: At the World Health Assembly in May, you asked member states to increase their contributions by 5%; they rejected that. Are they keeping WHO from fulfilling its purpose?

A: I'm the equivalent of the CEO of a company. So member states are like the board members. I said to them: "If you want

WHO to be strong and fit for purpose, keep your promises. Put your money where your mouth is." But many governments support a zero nominal-growth policy [for their contributions]. Maintaining that policy for 10 years has reduced the purchasing power of my budget by about one-third.

Q: You have advocated the idea of "white helmets," teams of health workers ready to be deployed in a crisis. What exactly are your plans?

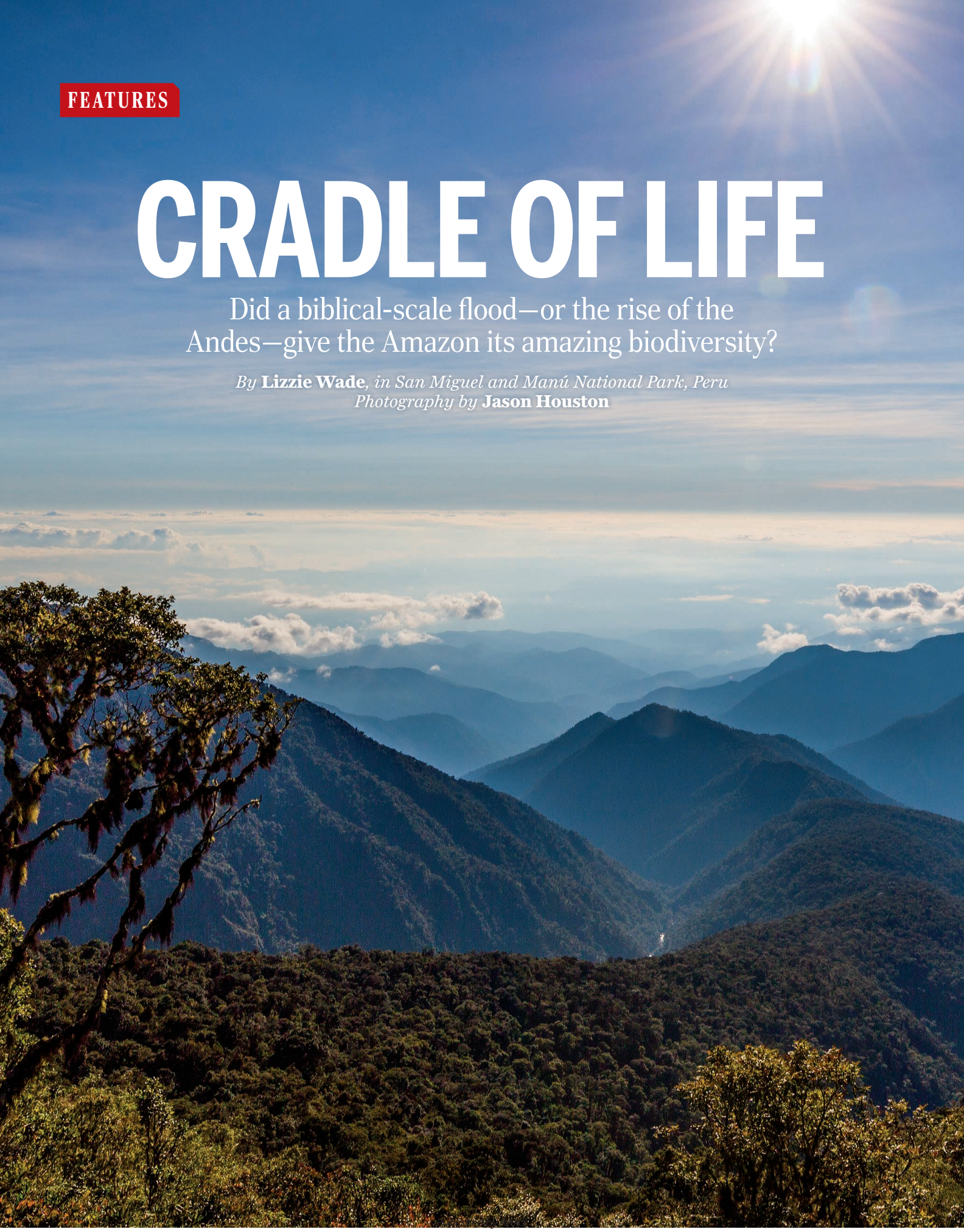
A: WHO is good in mobilizing support. But if that support is not prepared in advance, you cannot mobilize. Every country must have a rapid response team: epidemiologists, public health experts, clinicians, information specialists. When they are trained together, when they exercise together, when they mobilize as a team, they can hit the ground running. The idea is to have a core capacity at WHO. If the outbreak is small, we manage on our own. But if it is beyond our capacity, I mobilize all these pretrained teams. WHO should be the conductor of an orchestra.

Q: One proposed reform is restricting the director-general to just one 7-year term in office instead of two 5-year terms. Without the need to be re-elected, a director might find it easier to criticize member countries. Is that a good idea?

A: I have told member states that it's an idea they should consider. This job is getting more and more political and more and more intense. Can you imagine 10 years in this kind of position, where you have 194 bosses? And they do not necessarily agree on the difficult issues. I don't want to sound ungrateful. I feel privileged to be given the chance of serving the organization. But many countries behave like a visitor to WHO, not a shareholder. With the exception of a few countries, like Germany, the U.S., and the U.K., they are not serious in managing the organization. ... But the Ebola crisis has given me another opportunity to fast-track reforms. No more discussion. I keep telling people: I only have 21 months.

Q: At the G7 meeting of health ministers, you also talked about antimicrobial resistance. What did you say?

A: Governments need to pay attention. We are seeing more and more drug-resistant disease and the drug pipeline is empty. The last group of antibiotics we have was developed almost 30 years ago. If we run out of antibiotics, we are going into an era where simple infections can kill people again. ... Doctors should not prescribe antibiotics if they are not needed. Animal sector: Don't use them if they are not needed. ■



FEATURES

CRADLE OF LIFE

Did a biblical-scale flood—or the rise of the Andes—give the Amazon its amazing biodiversity?

By **Lizzie Wade**, *in San Miguel and Manú National Park, Peru*

Photography by **Jason Houston**

Trudging along the bank of a shallow creek in the Peruvian Amazon, Catherine Rigsby sinks knee-deep in mud. She calmly shimmies out of the muck, then grabs a branch lying on the bank for a walking stick. As stingless bees buzz around her head and macaws screech in the trees above, the sedimentologist gingerly resumes her search for a rainforest rarity: exposed rock. One of the few outcrops in this corner of the Amazon—a day's boat ride up the Manú River from the nearest town—is around here somewhere.

"I think we're in luck!" Sage Wagner, Rigsby's graduate student, calls from around a bend in the creek. Catching up, Rigsby, who splits her time at East Carolina University in Greenville and Yachay Tech University in San Miguel de Urcuquí, Ecuador, faces her quarry: a modest wall of sediment, about as high as she can reach. Deposited layer by layer some 9 million years ago, the outcrop holds clues to an enduring riddle: What gave rise to the Amazon rainforest's staggering biodiversity?

The western Amazon, which includes parts of Peru, Ecuador, Colombia, and northwestern Brazil, "is the most diverse region in the world in terms of plants," says Christopher Dick, an evolutionary biologist at the University of Michigan, Ann Arbor. "We have about 300 tree species in eastern North America. In the western Amazon, we have 300 tree species in a single hectare." And plant diversity is just part of the picture. All told, the Amazon Basin, a 6.7-million-square-kilometer area extending through Brazil all the way to the Atlantic, is home to 10% of the world's known species.

Scientists agree that the rich biodiversity springs from the convulsive geological changes that the western Amazon has experienced—mountains rising, coasts shifting, rivers changing course. By fragmenting and transforming habitats, these landscape changes would have driven bursts of speciation. But the experts differ, often vehemently, about just what form those upheavals took and which of them supercharged Amazonian speciation.

The prevailing scenario invokes an incursion of the Caribbean Sea into the South American continent that ended less than 10 million years ago, creating a vast wetland studded with islands. Organisms would have had to adapt to a patchwork of biomes that varied between salty and fresh, aquatic and terrestrial—driving up diversity.

To advocates of this scenario, fossils of plankton, mollusks, and marine fish as

well as present-day species like Amazon river dolphins provide powerful support. "I'm 100% positive" that at least twice in South America's long history, the Caribbean breached the continent's northern coast and poured into the western Amazon, 2000 kilometers away, says Carlos Jaramillo, a paleontologist at the Smithsonian Tropical Research Institute in Panama City.

But Rigsby's outcrop, which formed during the putative Caribbean invasion, challenges that picture because it suggests that the environment then was very much like today's. The 9-million-year-old sediment—coarse at creek level—becomes finer grained as Rigsby traces the layer upward. Half a meter or so up, the grains switch back to coarse, and the pattern repeats. Rigsby suspects she's looking at the traces of repeated river floods like those that occur today. The modern floods leave the same sedimentary signature of coarse grains giving way to fine grains as the waters subside.

Based on this outcrop and other geological evidence, Rigsby and others argue that the wellspring of the Amazon's extraordinary diversity was a much earlier event. Their favored candidate: the early uplift of the Andes on the basin's western edge, which began fitfully at least 65 million years ago. As species adapted to narrow ecological niches along the mountainsides, these researchers say, biodiversity soared and new species spilled from the Andes into the Amazonian rainforest.

With Dick and 18 other colleagues, Rigsby traveled to Peru in July in search of geological and biological data that could test their alternative theory about the Amazon's evolution. They examined not just rocks and fossils but also living plants, whose genomes hold clues to when and where Amazonian flora diversified. "We're going to get this huge amount of [genetic] data that, if we filter it correctly, can give us a lot of environmental history, geological history, climate history," says one of the team's leaders, Paul Baker, a geologist at Yachay Tech and at Duke University in Durham, North Carolina.

Other teams are also rising to this grand challenge of biogeography. CLIM-AMAZON, a joint European and Brazilian project, is puzzling out the sedimentary history of the Amazon Basin to trace ancient water bodies. And a group led by Joel Cracraft, an ornithologist at the American Museum of Natural History in New York City, and Lúcia Lohmann, a botanist at the University of São Paulo in Brazil, is mapping the ranges of Amazonian birds, butterflies, primates, and two plant families to understand how

ONLINE

View two videos online at http://scim.ag/vid_6260.

Listen to a podcast with author Lizzy Wade at http://scim.ag/pod_6260.

The foothills of the Peruvian Andes are home to some of the most diverse cloud forests in the world.



Graduate students Federico Moreno and Lauren Gonzalez sample sediment from a 9-million-year-old outcrop along the Manú River in Peru. Catherine Rigsby (pictured below) believes the sediments were left behind by a primeval river, pointing to an ancient environment very much like today's Amazon Basin.

the basin's history shaped its biogeography (*Science*, 13 July 2013, p. 234). All three teams will present findings next week at the Geological Society of America's annual meeting in Baltimore, Maryland.

The multidisciplinary synergy is "accelerating our view of Amazonia," Cracraft says. "We are on the cusp of understanding how this large-scale diversity happened."

FORTY YEARS AGO, scientists thought they had the Amazon all figured out. In a 1969 *Science* paper, ornithologist Jürgen Haffer mapped the distributions of Amazonian bird species and identified patches of high diversity (11 July 1969, p. 131). Those hotspots, he concluded, were the legacy of the ice ages. When temperatures across the world fell during the Pleistocene Epoch, starting about 2 million years ago, the Amazon dried out, he argued. Most of the basin became grassland, with pockets of rainforest hanging on in the most humid areas. Isolated in forest refuges, life forms diverged into new species. When warmer, wetter conditions returned, the forest expanded to cover the basin once again, bringing all those new species into contact. According to Haffer, this cycle repeated itself several times during the Pleistocene and the cur-

rent Holocene Epoch—revving up speciation each time. The refuges would stand out today as diversity hotspots within the larger forest—exactly what he saw in his bird data.

"It's not a bad idea," Dick says. But Haffer's hypothesis crumbled when a 1990 *Nature* paper showed that the biodiversity hotspots were illusions—the inevitable result of scientists collecting in the same eas-

ily accessible areas of rainforest over and over again. Meanwhile, pollen cores and isotope studies revealed that climate cycles had never turned most of the Amazon into arid grassland. "In fact, in the South American tropics, [the global ice ages] were wet," says Sherilyn Fritz, a paleoclimatologist at the University of Nebraska, Lincoln, and co-leader on Baker's project. "In a simple sense, the Haffer hypothesis was wrong." Scientists were left scrambling for a new idea to explain the Amazon's biodiversity.

That's when Carina Hoorn came along. Then a graduate student, the young geologist and palynologist was tromping around the western Amazon, building a picture of what the area looked like long before the Pleistocene. Millions of years earlier, in the Miocene Epoch—from 23 million to 5 million years ago—"there [was already] a highly diverse rainforest out there," she says.

Hoorn knew that for more than a century, scientists had been finding fossil mollusk shells in the western Amazon



that looked strikingly like ocean species. But she couldn't quite bring herself to believe that the Caribbean had reached so far inland. Then the fossilized remnants of foraminifera—single-celled organisms that live mostly in the sea—started showing up in her Miocene sediment samples from Peru, Colombia, and northwestern Brazil. “That’s what did it for me,” recalls Hoorn, now a researcher at the University of Amsterdam in the Netherlands and a leader of CLIM-AMAZON. Her inland samples also yielded pollen from mangroves, a tree that today grows in saltwater along tropical coasts.

“In the beginning I thought it was just a flooding event,” she says: a one-time rush of seawater into an inland region dominated by freshwater rivers and lakes. But the deeper she delved, the more she was convinced that the presence of saltwater was “rather common.”

She now pictures the western Amazon during the Miocene as an estuarine environment subject to saltwater incursions. Forested islands may have arisen, separating terrestrial populations and spurring speciation. Meanwhile, the seawater brought with it the marine ancestors of several river species found in Amazonia today, including dolphins and stingrays, says Nathan Lovejoy, an evolutionary biologist at the University of Toronto, Scarborough, in Canada. Still more signs of an ancient marine ecosystem came this summer when Pierre-Olivier Antoine, a paleontologist at the University of Montpellier in France, uncovered thousands of giant oysters, as well as fossil sawfish and stingrays. These scientists say that the estuary system—known as the Pebas wetland—endured for most of the Miocene.

Around the same time, however, the other potential driver of Amazonian speciation was stirring. The Andes were rising in fits and starts—and once that great wall was in place, South America would never be the same.

OUTSIDE THE MINING TOWN of San Miguel, 4000 meters up on Peru’s Altiplano, Carmala Garziona gasps for air. Armed with a set of GPS coordinates, Garziona, a geologist at the University of Rochester in New York, and her student, Federico Moreno, are leading the visiting researchers to an unmistakable relic of the Andes’ dynamic past.

After a breathless 3-kilometer hike, they find their treasure: a petrified tree trunk lying incongruously on the grassy plain. The cellular structure of its wood shows it is a member of the legume family, and the age of nearby limestone outcrops suggest the tree

is about 10 million years old. “Nothing like it lives at these altitudes,” Garziona says—which means that the forest of which the tree is a relic grew at a much lower altitude. The finding jibes with her earlier studies of chemical traces left in ancient soils by rainwater, which suggest that between 18 million and 9 million years ago, the Altiplano was about half as high as it is today.

In Hoorn’s scenario, the rise of the Andes to their full height between about 10 million and 5 million years ago spelled the end of the Caribbean incursions (*Science*, 12 November 2010, p. 927). Water vapor flowing across South America from the Atlantic couldn’t surmount the mountains and fell as rain in the western Amazon. Fresh water poured down the

ago, as Hoorn’s model assumes. But the story’s real beginning was much earlier, Baker says: the rise of the West Andes, a string of volcanoes that runs down South America’s Pacific coast.

The West Andes’ geological history is shrouded in mystery. Unlike the mountains of the East Andes, the volcanoes do not contain any ancient soils that Garziona uses to reconstruct environments long past. But the landscape itself gives a clue to the West Andes’ age. As the mountain belt rose, Garziona says, its weight depressed the nearby continental crust, creating a broad “foreland basin” in what is now central Peru. The basin filled with sediment; later mountain-building episodes thrust it upward to form the Altiplano and the East



Nothing like this fossilized tree grows on the Andean Altiplano today. When this member of the legume family was alive 10 million years ago, the mountains were barely half their current height.

eastern flank of the Andes, flushing out saltwater. The surge also eroded nutrient-rich sediments and carried them to the lowlands, enriching the soil of western Amazonia. The northern seaway connection to the Caribbean closed as the flow of water and sediments plowed eastward to the Atlantic. They broke through to the sea about 7 million years ago, and the modern Amazon River was born.

The notion of Miocene marine incursions ended by a burst of Andean uplift beginning 10 million years ago “is a beautiful story. It’s just, I think, completely wrong,” Baker says. Findings like the petrified tree leave little doubt that the East Andes shot up between 10 million and 5 million years

ago. From the age of the foreland basin and its sediments—now twisted inside the mountains and across the Altiplano—geologists estimate that the West Andes had risen to an elevation of some 3 kilometers by 53 million years ago, Garziona says.

To Baker, that means the Andean-driven processes—from speciation on the slopes to trapping moisture over the Amazon to pushing freshwater rivers east—could have started long before 10 million years ago, the date Hoorn and others propose. When the mountains rose, the burst of new habitats in both highlands and lowlands was as extreme as the geological transformation, Baker and others say. “If you had to point to one single event to explain the Neotropics’

biodiversity, I think it was the uplift of the Andes,” says Alexandre Antonelli, an evolutionary biologist at the University of Gothenburg in Sweden.

THE RELATION BETWEEN topography and species richness is vivid as Baker’s group leaves the Altiplano and, following an old Incan trail, descends the Andes’ eastern flank into the cloud forest. At about 3500 meters, the grassland gives way to trees draped in moss so dense it forms a tunnel around the path. At 2500 meters, the moss thins and woody vines snake through the trees. Lower still, the forest floor is lush with ferns. “There are no species that have ranges that extend the entire gradient,” says Miles Silman of Wake Forest University in Winston-Salem, North Carolina, an ecologist who studies cloud-forest trees. Just moving up and down the mountain, he says, “you accrue a lot of diversity.” The cloud forest is the roiling core where all these species mix, mingle, compete, and diversify, he says. “It’s the hottest of all biodiversity hotspots.”

Not only is there no need to invoke a Caribbean invasion to explain present-day diversity, it never actually happened, Baker and others contend. Marine incursions are “a pretty complicated story,” Rigsby says. “A complicated story requires a lot of data to back it up. And the data are just

not there.” The sedimentary outcrop in the eastern Peru, recording river floods like those of today, is one strike against the story, she says. And there are others.

Scientists who support the marine incursion theory “use proxies of things they think are only found in marine systems,” Dick says. “But all of the proxies are becoming, on inspection, weaker and weaker.” Many of Hoorn’s foraminifera species, for example, are found in water with a wide range of salinities, including fresh water. As for marine-derived species like the dolphins and stingrays in today’s Amazon River, it’s possible that their ancestors lived near the mouths of rivers and ventured upstream gradually, adapting to fresh water along the way, Baker says.

Geochemical clues also hint that western Amazonia in the Miocene may have been a freshwater system. Werner Piller, a paleontologist at the University of Graz in Austria, studied fossilized ostracods—small crustaceans with a clamlike shell—from the western part of the Brazilian Amazon. By analyzing the ratio of oxygen isotopes in the shells, Piller could tell whether they formed in saltwater or fresh water. “We get

“We are on the cusp of understanding how this large-scale diversity happened.”

Joel Cracraft,
American Museum of
Natural History

a clear freshwater signal,” he says.

In her own work, Hoorn says she has examined “meters and meters of sediment that look purely freshwater,” with vestiges of saltwater species like plankton and mangrove pollen limited to just a centimeter or two. “It’s a mixed message in the

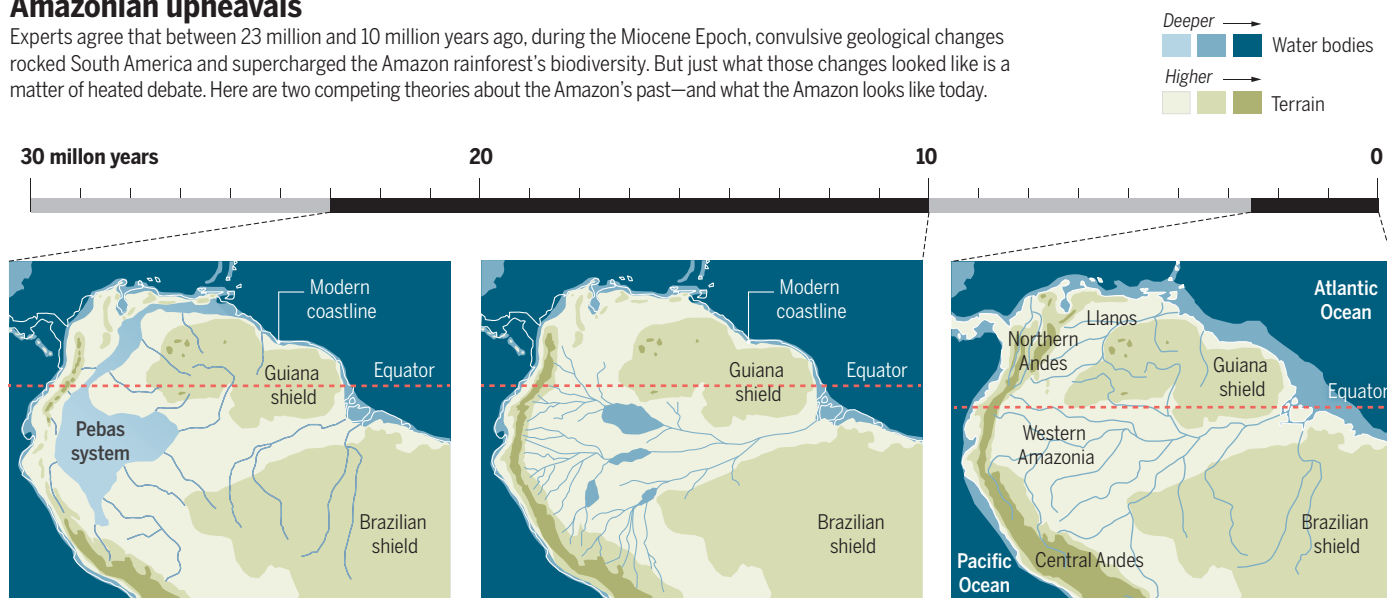
sediments”—one she admits can be confusing. But it’s also what she would expect to see in an estuary created by marine incursions: a shifting pattern of freshwater and saltwater dominance.

Lovejoy agrees, noting that the closest marine relatives of many Amazonian fish species, including freshwater stingrays, live in the Caribbean along the northern coast of South America—just where the marine incursions are thought to have originated. What’s more, their family trees suggest, he says, that “those [marine] lineages have only moved into the Amazon once or maybe twice,” a hint that they invaded the Amazon during a marine incursion rather than slowly colonizing fresh water from river mouths.

“There are really a lot of different types of evidence,” Hoorn says. “You cannot disregard that.”

Amazonian upheavals

Experts agree that between 23 million and 10 million years ago, during the Miocene Epoch, convulsive geological changes rocked South America and supercharged the Amazon rainforest’s biodiversity. But just what those changes looked like is a matter of heated debate. Here are two competing theories about the Amazon’s past—and what the Amazon looks like today.



Marine incursion theory

Traces of plankton and marine fossils suggest that during the Miocene, the Caribbean Sea invaded South America. The western Amazon became a brackish estuary known as the Pebas system. The shifting patchwork of terrestrial, saltwater, and freshwater environments drove speciation.

Ancient Andes theory

Sediments left behind by primeval rivers and chemical analyses of fossil shells cast doubt on marine incursions. Instead, high peaks in the western Andes trapped precipitation, fed freshwater rivers, and created an abundance of mountainside habitats that pumped new species into the rainforest.

Current state of Amazon Basin

Cloud forests connect Andean peaks to lowland rainforest. Fresh water pours off the 4-kilometer-high mountains, feeding the Amazon River and its tributaries. The 6.7-million-square-kilometer basin is home to 10% of the world’s known species, making it one of the most species-rich regions on the planet.

CRACK! CRRRRRACK! Christopher Dick pulls a rope to snap shut a lopping shear at the end of a long pole. The blades bite into a branch of a small, spine-covered tree in the old growth rainforest around Cocha Cashu, an isolated research station in Manú National Park. The branch snaps and plunges into a palm frond below. Dick shakes it to the ground and targets the freshest looking leaf, writing the date, sample number, and the code for its species on it with a Sharpie. He snips out this smaller section and bags it for DNA analysis back at the University of Michigan.

Dick hopes to find clues to the origins of today's species by decoding the environmental history embedded in their genes. The spines covering the tree he has sampled identify it as *Poulsenia armata*. *Poulsenia* grows on both sides of the Andes, in the Amazon as well as in western Ecuador and even as far north as Panama. But the species isn't a restless pioneer: It tends to stay put in the rainforest, dispersing its seeds nearby with the help of animals. There's no way it could climb over the Andes, or even move around them, once the mountains were at their full height, Dick says. In all likelihood, *Poulsenia* was already growing from Panama to Peru before the Andes rose.

The rising mountains separated populations of *Poulsenia*, setting them on different evolutionary paths. Since then, each *Poulsenia* population has accumulated random mutations in its DNA. By tallying up those changes, Dick can estimate when populations on opposite sides of the mountain range formed one continuous group—and, therefore, estimate the age of the peaks. "Anytime you have a geographic barrier, [*Poulsenia*] reflects how old that barrier is in the DNA," he explains.

When Dick did this for *Poulsenia* samples from opposite sides of Ecuador, he found that the populations had a common ancestor about 9 million years ago—about the time Garzone says the Andes were going through their most recent growth spurt. Now, Dick wants to compare the Ecuadorian populations with *Poulsenia* samples from Peru. He hopes his results will yield clues to the timing and extent of the Andes' earlier growth spurts—and help to test ideas about the role they could have played in speciation. "Geogenomics," as Baker calls it, can be a particularly useful approach in a place like the Amazon, where outcrops are scarce.

Baker has a plan for expanding geological sampling as well. He's gathering funding for an international project that would drill five or more sediment cores from the mouth of the Amazon River to the base of the Andes. That, Hoorn says, would give scientists



In a remote rainforest in Manú National Park, Christopher Dick clips leaves from the tree *Poulsenia armata*. He hopes to find signs of ancient geological changes lodged in the tree's genome.

an unbroken look at the landscape over the course of 65 million years or more, rather than forcing them to "piece it all together" from disparate and hard-to-date samples. The drilling project "is essential," Cracraft agrees. "It will do more to help reconstruct the environmental and hydrological history of Amazonia than anything we've done previously."

But for now, Baker and his colleagues

are making do with the precious snippets of Amazonian history they've gathered on this trip: armored leaves, a rare outcrop, and a petrified tree. They will resume their quest for the origins of its staggering diversity back in their labs. For now, as they chug up the Manú River and out of the jungle, they're content to watch the Amazon's landscape—at once dynamic and seemingly eternal—glide by. ■

INSIGHTS



BOOKS *et al.*

SCIENCE FICTION

Adventures in time and space

From the robots of Isaac Asimov to the vision of cyberspace conjured by William Gibson, science fiction can both inspire scientific advancement and offer readers a glimpse into how today's technologies might fit into the world of tomorrow. Not to mention the fact that the fantastical story lines for which the genre is well known make for awfully fun reading. The prestigious Nebula and Hugo awards recognize outstanding new works in science fiction and fantasy, as nominated and chosen by members of the Science Fiction and Fantasy Writers of America and World Science Fiction Society, respectively. Here, we review the most recent winners and finalists for best novel for each of these awards.

2014 NEBULA AWARD WINNER

Into the unknown

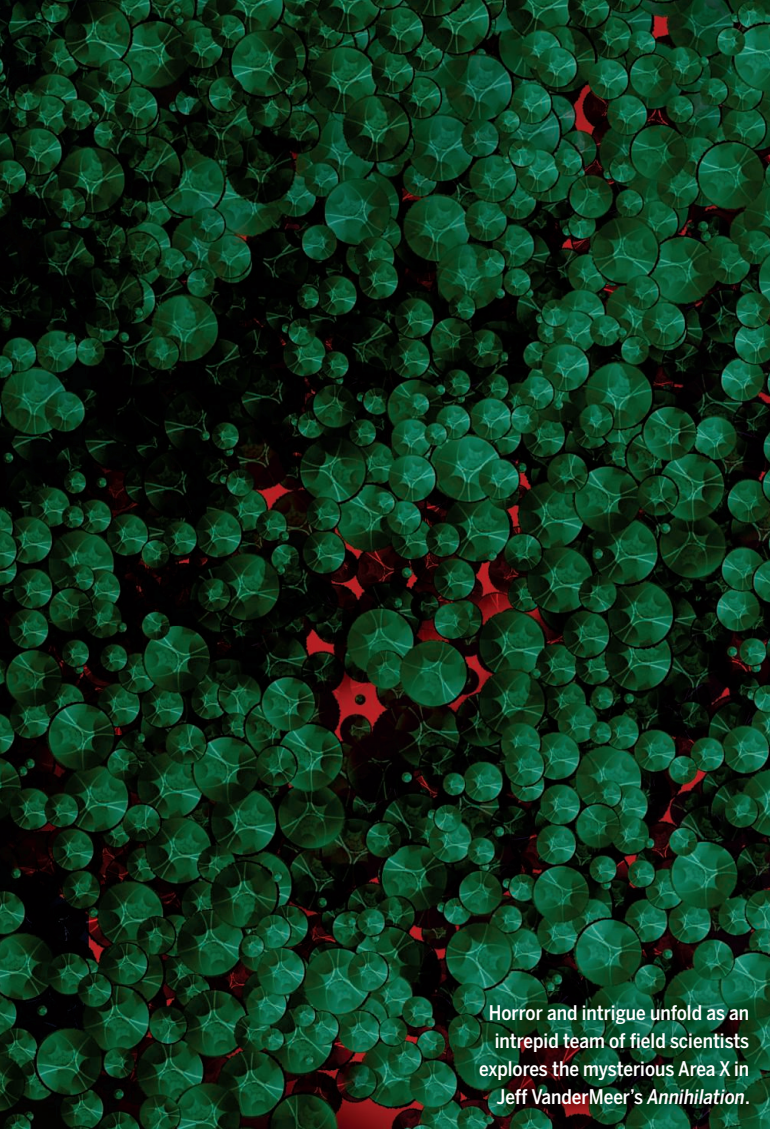
Reviewed by **David S. Nelson**

Take a dangerous, mysterious, uninhabited locale; send in four scientists to study it; add exotic life-forms, lots of self-doubt and paranoia, and some bits of philosophy, and you get *Annihilation*, the first book in Jeff VanderMeer's Southern Reach Trilogy.

As the story begins, we learn that the government of the so-called

"Southern Reach" has sent a number of teams to explore and report back on the forbidden "Area X." Do they get there by teleportation, time travel, or simply by strolling through some picturesque woods? We are not told, and the explorers themselves do not remember. What we do know is that no mission has returned sound of mind and body, and many agents have been killed. The current expedition is composed of four female scientists: the Surveyor, the Anthropologist, the

*The reviewer is the winner of the 2015 Bulwer-Lytton Fiction Contest for the fantasy genre.
E-mail: dsn1nelson@aol.com*



Horror and intrigue unfold as an intrepid team of field scientists explores the mysterious Area X in Jeff VanderMeer's *Annihilation*.

NEBULA FINALISTS

Coming Home

Jack McDevitt

Ace, 2014. 366 pp.



Coming Home is a bit like an archaeological dig for the reader who must piece together bits and pieces of information to understand why Earth's civilization, which had been going through a Golden Age, fell apart. The book follows Alex Benedict, an antiquities dealer, and his assistant, Chase Kolpath, who are in the midst of looking for the remains of ancient Earth when they are caught up in a mystery: A colleague has committed suicide, and a priceless artifact is discovered in his possession. To add to the book's suspense, the story also features a global effort racing to rescue the passengers of an interstellar transport ship that has been caught in a time/space warp. McDevitt's attention to detail makes this a good read. —*Barbara Jasny*

Trial by Fire

Charles E. Gannon

Baen, 2014. 640 pp.



After a failed exosapient convocation, human planets are invaded by alien beings. Overrun by superior technology, humanity comes under the control of two oddly aligned species working with corrupt leaders and corporations on Earth. Caine Riordan, a journalist turned operative turned diplomat, becomes the focal point of the human response as he tries to discover the reason for the invasion. Although the book is plagued with too many minor characters and combat scenes, Gannon creates intricate, intertwined story lines that match the complexity and cunning interspecies relationships. Caine's gradual extraction of the truth brings many interesting discoveries. —*Marc Lavine*

Psychologist (the putative leader), and the Biologist (our narrator).

The team's first major discovery is an enormous underground structure very close to base camp that is chock-full of slime, fungi, unknown microfauna, mysterious undulating graffiti on the old stone walls, odd sounds and smells, and spore-blasting plants. As the story advances, we are led to believe that deep down the structure's seemingly interminable stairways lurks something quite strange and probably dangerous. It is not long before fear and paranoia set in, and any sense of cohesion or mission is lost when one of the scientists goes missing and another is found murdered.

Down by half, the remaining pair split up and individually, guardedly, trek to the lighthouse on the beach a half-day's hike away. They knew plenty about the lighthouse from their mission training; their trainers had told them that it was an important focal point that offered clues to the strangeness and degeneration of Area X. But we soon learn that there's a lot more to discover. Fortified and battle-scarred, the lighthouse holds the discarded journals of previous expeditions, which yield alarming information about the failure of earlier missions and the threats they faced.

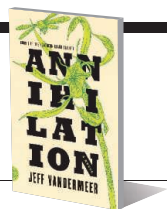
Independent and introspective, the Biologist begins to believe that her perceptions are being altered and that she is metamorphosing (into what, she's not sure) as a result of inhaling spores in the underground structure. Cellular samples seem to change their identity when she looks at them under the microscope, and the wildlife she encounters begins to exhibit some decidedly human qualities.

Annihilation

Jeff VanderMeer

FSG Originals, 2014.

207 pp.



Summoning the nerve to head back to base camp, she ventures on alone into the depths of the otherworldly underground edifice. There is indeed something down there, something of power, something more felt than seen, something drawing her on. Perhaps all life-forms in Area X undergo metamorphosis, evolution, repurposing; perhaps we all do.

When it comes to getting the science right, the book could have done a better job, and at times the story seems a bit loosely jointed. I would expect an experienced field biologist to know that a snake can be venomous, not poisonous, for example, and that both moss and fungi are eukaryotes. In an interview with *The Guardian*, the author said his series is "an examination of the dysfunction and absurdity found in human-created systems," so perhaps some of the mysteries will be clarified in books two and three of the series.

VanderMeer is clearly trying for a mood of spookiness, dread, and unease, and he achieves it near the end of the book, as exemplified in the following passage: "it became an overwhelming *hugeness* in my battered vision, seeming to rise and keep rising as it leapt toward me. The shape spread until it was even where it was not, or *should not have been*." Perhaps this reviewer has read too much Lovecraft and Bierce, but I rarely got the delicious sense of creepiness I sought. Nonetheless, it is the 2014 winner of the prestigious Nebula award for the best science fiction novel and clearly has its fans among science fiction writers and the public.

10.1126/science.aad5282



2015 HUGO AWARD WINNER (AND DUAL FINALIST)

Culture shock

Reviewed by Yevgeniya Nusinovich

From the very first scenes depicting street violence precipitated by the Chinese Cultural Revolution of the 1960s, it is clear that *The Three-Body Problem* is no ordinary science fiction story. Within a few pages, the scene shifts to a public rally on the outskirts of town, where a scientist is being verbally and physically abused for refusing to renounce his work and kowtow to the Communist government. The opening of the book paints a portrait of a dark period in Chinese history where there are no clear heroes and no real hope, evoking some of the dramatic scenes from *Les Misérables* but without the hopefulness and idealism. What emerges from this darkness is a futuristic masterpiece weaving together hard science and exis-

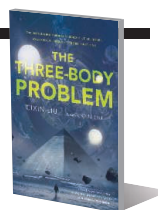
tential questions in a story full of unexpected twists.

The plot of the book develops through a series of seemingly unrelated vignettes, some of which are set in the 1960s, during the Chinese Cultural Revolution, others in modern-day China, and yet others inside an unusually detailed and realistic video game called “Three Body,” in which players struggle to stay alive in a world where day length varies unpredictably and the temperature changes from equatorial heat to deadly cold at a moment’s notice. The principal characters in the book are all physicists, although their actions and moral choices are shaped more by their circumstances than by their scientific backgrounds. While the characters in the historical setting struggle to survive the revolution without being exiled or killed for their “reactionary doctrines,” the modern characters find themselves facing a more mysterious, but no less deadly, threat.

As the three settings become progressively more connected throughout the story, readers

The Three-Body Problem

Cixin Liu,
translated by Ken Liu
Tor, 2014. 399 pp.



Set against the backdrop of China's Cultural Revolution, *The Three-Body Problem* is a rich blend of world history and speculative fiction.

may find themselves trying to guess which characters are good or evil and what they will do next. However, at least for this reader, such guesses often turned out to be wrong. The unexpected plot twists did not feel forced or gimmicky but did leave me feeling like I was traveling in a foreign city where there were no familiar landmarks and anything could happen. Some of this can undoubtedly be attributed to the author's creativity, but I could not help wondering whether readers who are well versed in Chinese culture and conventions would have found the plot to be more familiar and predictable.

In addition to the many personalities and historical events, science, primarily physics, is a constant presence throughout the book. Like the Chinese history, the concepts are explained clearly enough for a nonphysicist to follow the story line without difficulty. The physical and mathematical concepts that form the foundation of the story appear to be scientifically sound and logically connected, as

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HUGO FINALISTS

The Dark Between the Stars

Kevin J. Anderson

Tor Books, 2015. 782 pp.



The galaxy is a big place, and *The Dark Between the Stars*, the first book in a three-volume series, seems to try to duplicate its dimension in 782 pages. The story is an extravagant science fiction epic that finds humankind in an alliance with an alien race that only recently had been its adversary, as the two forces unite to fight a powerful new enemy. The book is chock-full of characters, a passel of exotic species and races, strange matter, mysterious powers, deadly plagues, and conflict. The result is a challenging amalgam of story lines that are just the beginning of a wider narrative arc that, for now, remains cast in shadow. —Jesse H. Smith

Skin Game

Jim Butcher

Roc Books, 2014. 608 pp.



Skin Game continues the adventures of Harry Dresden, private investigator: a wise-cracking urban wizard in an alternate universe in which magic is real and humans coexist with supernatural entities. As the novel opens, Harry finds himself pressured into joining a team of villains on a dangerous mission to raid Hades' ultrasecure vault. He must outsmart its notoriously treacherous leader without putting anyone else in harm's way. Several of the plot twists hinge on the characters' calculated approach to magical elements, which strictly obey certain basic principles and interact sensibly with the laws of physics. —Aaron M. Ucko

DUAL FINALISTS

The Goblin Emperor

Katherine Addison

Tor Books, 2014. 510 pp.



The emperor's fourth son, Maia, is the result of a single union from a political marriage with a goblin. Maia was ignored and unfavored by his father, but upon the death of his male family, he is thrust from exile into ruling the elven kingdom. As a youngster of 18, Maia must seize the reins of power quickly and hold fast. Although the story of his learning to rule generally flows well, it is not a book with much action. Rather, this novel examines how an outsider can learn to use power wisely. —Laura Zahn

Ancillary Sword

Ann Leckie

Orbit, 2014. 391 pp.



Ancillary Sword, the second book in the Imperial Radch trilogy, gives us a deeper insight into the mind of the sentient starship known as *Justice of Toren*. Once an entity that replicated its artificial intelligence in a massive army of human soldiers—the ancillaries of the title—the troop carrier was destroyed, leaving only a single survivor. This human ancillary, Breg, the reluctant hero of the first book, is sent to an outpost of the Radch empire, where she must resolve several incipient ethnic conflicts that threaten the local status quo. The book is slower paced and more mannered than the first: the narrative strictly linear and the action of less consequence. Well written though it is, it feels like a set-up for a hopefully much more gripping finale. —Guy Riddihough

well as clearly explained. As a biologist, I was alarmed to see some implausible dangers attributed to genetically modified crops in one scene, but this was a minor point that did not take away from the story line or the beauty of the book.

Thanks to the efforts of the translator, Ken Liu, and a few judiciously interspersed footnotes, the historical context for the book is quite clear even to a reader who knows little about Chinese history. Furthermore, Liu was very effective at not only explaining all concepts as needed but also conveying a sense of Chinese culture and style in a way that felt fluid and natural.

In general, this novel should please fans of science fiction and suspense alike and will hold particular interest for readers who enjoy physics, mathematics, and/or history. On finishing the book, I found myself eagerly looking forward to reading the next part of this trilogy. *The Three-Body Problem* is highly deserving of the Hugo Award and represents a great book for introducing Chinese science fiction to a worldwide audience.

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GEOPHYSICS

Pinched topography initiates the critical zone

Geophysical imaging provides a clearer picture of how rock turns into soil

By Robert S. Anderson

In Earth sciences, the critical zone represents the intersection of the biosphere with the atmosphere, hydrosphere, and lithosphere (1, 2). The myriad interactions and feedbacks among these systems assure us of a world with considerable complexity, in which the critical zone varies in thickness, mineralogy, permeability (3), and structure of ecosystems (4). It is no wonder, then, that we lack a general theory of how the critical zone works. On page 534 of this issue, St. Clair *et al.* (5) argue that we must take the broadest possible view of, and acknowledge a role for, large-scale tectonic stresses in guiding the pattern of cracking of rock in the subsurface.

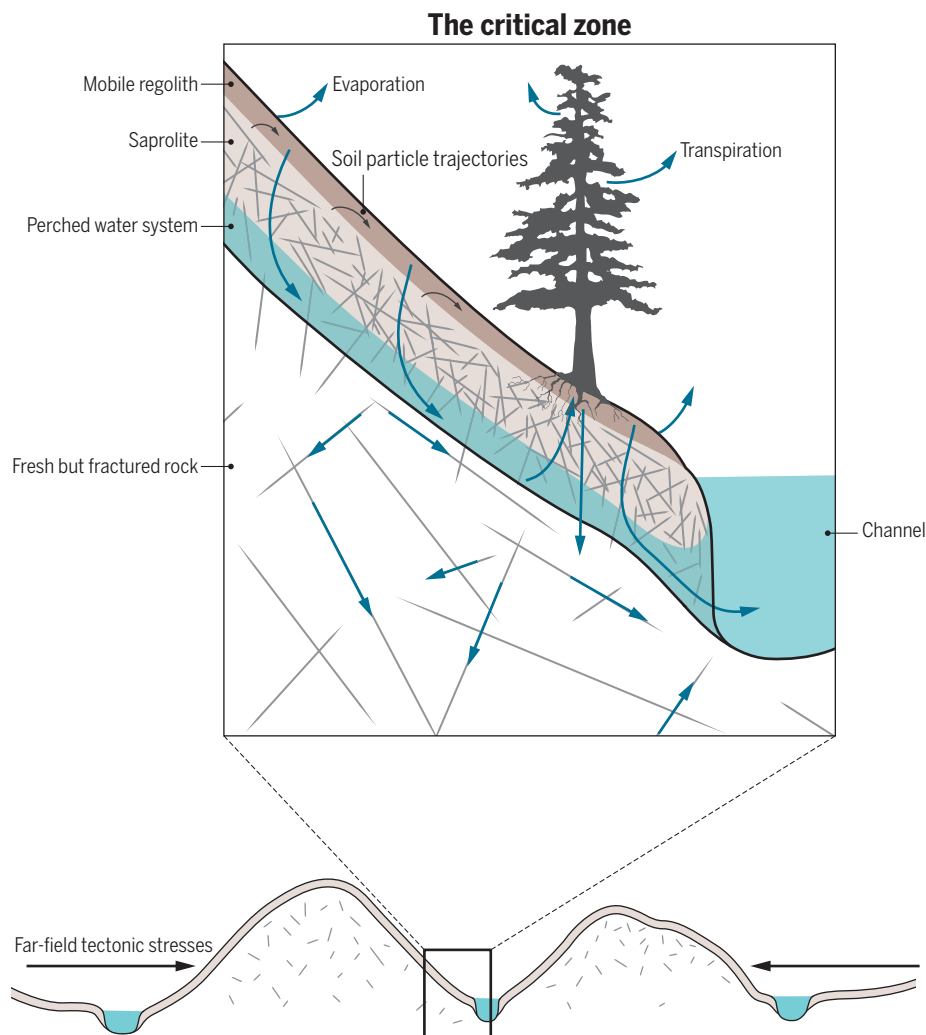
Consider a hillslope bounded by stream channels (see the figure). Rock is released as transportable particles into the soil on its surface, which then carries the particles through physical and biological transport processes to streams. Water, by contrast, is poured onto the landscape from above, either as rain or as snowmelt, with chemistry that is effectively distilled through evaporation from its source. The water travels downward through the soil, and through rock fractures that ultimately deliver it to the stream. As the water travels, interaction with the minerals of the soil and rock, catalyzed by biological interactions, both changes the strength, porosity, and permeability of the rock, and charges the water with ions that constitute the nutrient supply for plants. The generation of porosity transforms the rock into a substrate capable of sustaining an ecosystem, which in turn, through the action of roots, aids in the breakdown of rock (2, 3). These two trajectories, of rock particles and of water molecules, tangle in the critical zone, where their manifold interactions are indeed critical to life—hence the “critical zone.”

The most difficult of these processes to document are those that herald the arrival of rock into the surface environment and initiate its transformation from unweathered fresh rock. Any debate about the relative importance of processes tied to the surface, and those initiated at much

greater depths (6), in promoting the transformation of fresh rock must ultimately be informed by field data.

Seismic refraction and electrical resistivity methods, often used in deep crustal studies, are seeing greater use in shallow settings (7, 8). St. Clair *et al.* undertook a campaign-style geophysical characterization of the subsurface across ridge-channel pairs in multiple landscapes. They leveraged the geologic and climatic diversity of accessible, well-studied sites within the U.S.

Critical Zone Observatory network. Using sites in the humid eastern United States and in the Rockies of the arid western United States, the team found surprisingly different patterns of seismic velocities across the ridge-slope-channel transects. In some sites, low seismic velocities, interpreted as high density of cracks in the subsurface, were confined to a thin surface-parallel layer. In others, the zone of low-velocity, cracked rock extends more deeply beneath the ridges than below the stream chan-



The critical zone. Transformation of fresh rock into soil involves cracking of the rock and chemical attack of its minerals. Coevolution of the permeability of the rock mass, the pattern of water flow (blue arrows), and the ecosystem constitute a complex system that varies in time and location as a result of rock type and climate. St. Clair *et al.* argue that the pattern of cracking of the rock as it nears the surface also depends on topographic stresses, reflecting the interplay between the topography itself and the far-field stresses that pinch the topography (arrows).

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nels, generating a “bowtie” image. These very different patterns of deep critical zone structure are not easily explained with climate. The authors used a numerical model of the state of stress in an elastic rock mass into which a landscape has been carved (9) to calculate the pattern of expected cracking of the rock. The topographic stresses arise from both the topography itself, and the far-field horizontal stresses imposed by the tectonic setting (arrows in figure) constrained by an existing world map of stresses. As the far-field stresses are increased, the pattern of expected cracking morphs from the surface-parallel to bowtie patterns, capturing both end-members of the observed seismic images. This is indeed an encouraging result.

Are we to believe their results? In many mountain ranges, the rock arriving in the near-surface zone is already riddled with flaws that have accumulated as it moved through the tectonic stress fields of present and past orogenies (10). To what degree does the presence of such preexisting flaws violate the assumption that the rock behaves as a uniform elastic medium? How well does the present state of stress reflect the long-term history of stress to which a rock has been subjected? One can also imagine situations in which other processes that generate near-surface cracks [for example, frost-cracking (11)], or that chemically weather the rock as it nears the surface (12), are instead the rate-limiting steps in damaging the rock.

Whatever the answers, the results reported by St. Clair *et al.* will challenge the broader community to entertain a role for the state of stress imposed by the topography itself and its tectonic setting. They have also demonstrated the utility of classical geophysical methods and of a network of sites to test their ideas. ■

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MICROBIOME

A unified initiative to harness Earth's microbiomes

Transition from description to causality and engineering

By A. P. Alivisatos,* M. J. Blaser, E. L. Brodie, M. Chun, J. L. Dangl, T. J. Donohue, P. C. Dorrestein, J. A. Gilbert, J. L. Green, J. K. Jansson, R. Knight, M. E. Maxon, M. J. McFall-Ngai, J. F. Miller,† K. S. Pollard, E. G. Ruby, S. A. Taha, Unified Microbiome Initiative Consortium

Despite their centrality to life on Earth, we know little about how microbes (1) interact with each other, their hosts, or their environment. Although DNA sequencing technologies have enabled a new view of the ubiquity and diversity of microorganisms, this has mainly yielded snapshots that shed limited light on microbial functions or community dynamics. Given that nearly every habitat and organism hosts a diverse constellation of micro-

POLICY organisms—its “microbiome”—such knowledge could transform our understanding of the world and launch innovations in agriculture, energy, health, the environment, and more (see the photo). We propose an interdisciplinary Unified Microbiome Initiative (UMI) to discover and advance tools to understand and harness the capabilities of Earth's microbial ecosystems. The impacts of oceans and soil microbes on atmospheric CO₂ are critical for understanding climate change (2). By manipulating interactions at the root-soil-microbe interface, we may reduce agricultural pesticide, fertilizer, and water use enrich marginal land and rehabilitate degraded soils. Microbes can degrade plant cell walls (for biofuels), and synthesize myriad small molecules for new bioproducts, including antibiotics (3). Restoring normal human microbial ecosystems can save lives [e.g., fecal microbiome transplantation for *Clostridium difficile* infections (4)]. Rational management of microbial communities in and around us has implications for asthma, diabetes, obesity, infectious diseases, psychiatric illnesses, and other afflictions (5, 6). The human microbiome is a target and a source for new drugs (7) and an essential tool for precision medicine (8).

The National Science Foundation's Microbial Observatories, the U.S. Department of Energy's Genomic Sciences program, the Na-

tional Institutes of Health's Human Microbiome Project, and other efforts in the United States and abroad have served as critical first steps in revealing the diversity of microbes and their communities. However, we lack many tools required to advance beyond descriptive approaches to studies that enable a mechanistic, predictive, and actionable understanding of global microbiome processes. Developing these tools requires new collaborations between physical, life, and biomedical sciences; engineering; and other disciplines.

AREAS OF EMPHASIS. A central purpose of the UMI is to develop cross-cutting platform technologies to accelerate basic discovery and translation to applications. We highlight key needs and opportunities.

Decrypting microbial genes and chemistries. Approaches for characterizing microbiomes increasingly rely on whole-community metagenomic sequencing, yet roughly half of the genes identified in these studies encode products of unknown function, and existing functional annotations are often incomplete or inaccurate (9). Technologies for resolving roles of uncharacterized genes with high

“we envision...evidence-based, model-informed microbiome management...”

throughput and high accuracy are needed. These approaches must integrate improved computational methods for in silico prediction of protein and RNA functions, rapid mutagenesis of model organisms or native strains under natural conditions, multi-omics and high-resolution phenotyping platforms to test functional predictions in vitro and in situ, and improved capture of information in the literature.

Deciphering chemistries of microbiomes is essential. In untargeted metabolomics studies using mass spectrometry, less than 2% of data can be matched to known chemical compounds, and only a fraction of those map to recognized biochemical pathways (10). Advances have been made in predicting structures from mass spectra, but improvements are needed in both in silico and physical

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technologies to illuminate the “dark matter” of microbial chemistries.

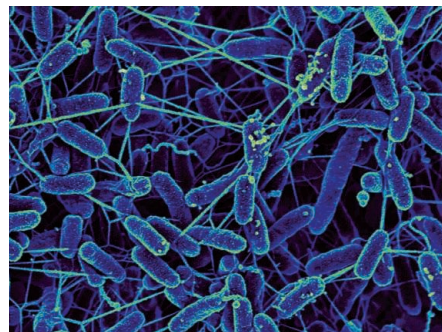
Cellular genomics and genome dynamics. Simply knowing which genes are present in a microbial population, without understanding their physical linkage, precludes organism-based insights into community function and dynamics. A transition from gene-centric to whole-genome-based analyses is vital and will require technologies capable of generating complete and assembled genomes from individual cells in complex microbiomes with high throughput, low cost, and minimal quantities of DNA. Advances are needed in long-read and single-cell sequencing platforms, improved algorithms for genome assembly, and comprehensive collections of reference genomes.

High-throughput, high-sensitivity multi-omics and visualization. Studies that integrate metagenomics, transcriptomics, proteomics, and metabolomics have been reported, but they are limited by coarse temporal and spatial scales and the absence of contextual information. Future discoveries will require new multimodal imaging capabilities that allow individual microbes—and their interactions, products, and identities—to be visualized within complex communities (11). Techniques that integrate high-resolution optical imaging with submicron-scale spectroscopy, and nondestructive nanoscale sensing platforms that allow longitudinal measurements, will help us understand how chemical conversations shape microbial communities and their environments.

Modeling and informatics. Comprehensive understanding of a microbial community can only be achieved by integrating imaging and multi-omics data sets with measurements of environmental or host parameters over relevant temporal and spatial scales. Adaptive models that capture the complexity of interactions from molecules to microbes, and communities to ecosystems, and new approaches for visualizing complex data sets in multiple dimensions, will contribute to a systems biology of microbiomes capable of yielding models with high predictive value. This will require new computational tools and innovations in mathematics, statistics, machine learning, and related fields. To ensure that data are openly available in a common format that can be processed by diverse computational tools, data commons, and standard languages for data reporting, such as those developed by the Genomic Standards Consortium (12), will be essential.

Perturbing communities in situ and tractable model systems. Transitioning microbiome research from a correlative science to

one based on experimental assessments of causality requires tools for manipulating microbial communities. Precision approaches are needed for stimulating, inhibiting, adding, removing, or altering microbes and their genes in situ, alone, or in combination and without cultivation. Potential tools include sequence-specific gene editing using CRISPR/Cas9 delivered by phage or conjugative elements (13), contractile nanotubes with strain-specific bactericidal activity (14), defined nutrient combinations based on modeled metabolic networks, and synthetic microbial consortia engineered to



Microbial community. *Shewanella oneidensis* with electron-conducting protein nanowires form an electric circuit to respire by transferring electrons to metal oxide.

disrupt or replace existing communities. Tractable model systems that approximate natural environments, including culture-based methods, and studies of naturally occurring microbiomes of low complexity such as those found in several insects, squid, and other organisms, will enable discoveries of mechanisms that drive interactions between microbes and their habitats (15).

IMPLEMENTATION. These goals are ambitious, but not beyond reach. Many tools we call for are extensions of existing technologies, albeit ones that will require ingenuity and resources to implement. Over the near term of 5 years, these tools could reorient the field from correlative studies to hypothesis-driven approaches capable of establishing precise causal relationships. Over a longer term of 10 years, we envision a leap toward predictive understanding that allows evidence-based, model-informed microbiome management and design.

Realizing the goals of the UMI will require a continuing and well-resourced public-private effort. Involving physical scientists, engineers, and others in an interdisciplinary initiative will lead to tool development and insights that have applications in different environments and beyond microbiome research. This creates the potential to accelerate and transform research supported by multiple government agencies, private

foundations, and industries, with anticipated economies of scale. Alignment of efforts of the many funders of microbiome-related research could leverage existing resources for greater yield, forge new funding approaches, amplify benefits of increased investment, and attract entities not yet involved in microbiome-related research.

Funding mechanisms will need to reflect the cross-cutting nature of the initiative. In addition to traditional agency-specific requests for proposals, multi-agency joint calls for development of broadly applicable tools could ensure coordination and availability of sufficient resources while reducing redundancy. These mechanisms should be designed to attract, train, and support diverse, multidisciplinary networks of scientists and engineers and to encourage disruptive ideas. Efforts should be made to identify microbiome-related translational opportunities and reduce barriers to industry participation.

The research community must help steer this effort by participating in the exchange between disciplines and by communicating insights and implications. The scientific community must also integrate ethicists, social scientists, regulators, and legal professionals at an early stage to ensure that risks associated with microbiome research are accurately assessed and proactively addressed.

As U.S. scientists, we call for a national initiative, but the challenge warrants a concerted global response to promote good practice and speed progress. Such an alliance could develop large-scale international collaborations and coordinate shared assets and consensus standards for global microbiome research. Fueled by the energy and vision of the scientific community and cross-cutting public and private partnerships, the UMI will lead to scientific insights, technological advances, and economic opportunities of lasting benefit to future generations. ■

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SUPPLEMENTARY MATERIALS

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See the supplementary materials for authors' affiliations.
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Randomness rules

Disorder can have dramatic effects on quantum transitions

By Nina Markovic

Phase transitions are perfect examples of physical phenomena for which statistical physics offers powerful predictions. Different types of phase transitions, ranging from liquid-vapor to ferromagnetic transitions, can be treated within the same theoretical framework. This yields useful expressions for characteristic physical properties of the system, such as resistivity, heat capacity, or free energy near the phase transition. The theoretical predictions can be strongly affected by random disorder, such as impurities or vacancies that are inevitably present in all real physical systems. In some systems, rare but large spatial regions are present in which there are no impurities. Such rare regions may be in a phase different from that of the bulk of the system and can dramatically alter the nature of the transition, causing certain physical properties of the system to diverge to infinity in the vicinity of the transition. These infinities are called Griffiths singularities (1) and can be expected to occur in a variety of systems, but they are not easily observed experimentally (2). On page 542 of this issue, Xing *et al.* (3) report the first experimental evidence of a Griffiths singularity near a quantum phase transition in a two-dimensional (2D) superconducting system.

What is a quantum phase transition? A familiar classical transition is the melting of a solid when the temperature of the system is increased. In contrast, quantum phase transitions occur at temperatures approaching absolute zero and are driven by some parameter of the system other than the temperature (4). A paradigmatic example is the superconductor-insulator transition driven by a magnetic field (5). In the absence of a magnetic field, the system is in the ordered superconducting phase and the resistivity is zero. As the magnetic field is increased, the phase fluctuations of the superconducting wave function disrupt the ordered phase at the quantum critical point, causing the system to become insulating.

Absolute zero temperature cannot be reached experimentally, but a signature of this phase transition can be observed at very low temperatures as a crossover at some critical magnetic field B_c that separates a region where the resistance decreases with

decreasing temperature (incipient superconductor) from the region where the resistance increases with decreasing temperature (incipient insulator).

As shown in the seminal work by Fisher (6), measurable physical quantities scale with the distance from the phase transition as $(B - B_c)/[T^{1/(vz)}]$, where v and z characterize the spatial and temporal divergence of the superconducting fluctuations near the critical point, respectively. The critical exponents v and z are universal, which means that they do not depend on the microscopic details of the system. This scaling behavior reflects a curious fact that a d -dimensional quantum system can be modeled by a $d + 1$ -dimensional classical system. The extra dimension is imaginary time, a quantity that is inversely proportional to temperature. Such a system has a finite size at nonzero temperatures, but it is infinite in the extra dimension at zero temperature.

How does random disorder affect the quantum phase transitions? The simplest type of disorder is represented by impuri-

ties and defects that do not change in time, dubbed “quenched” disorder. Quenched disorder can have dramatic consequences on the nature of the phase transition itself, but it is generally assumed that it does not appreciably alter either of the two bulk phases. Depending on the strength of the disorder, the quantum critical behavior can be essentially unaffected, it can be slightly smeared, or it can be completely obliterated.

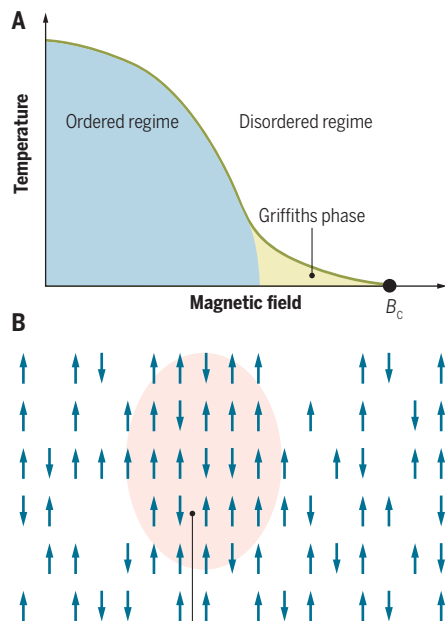
The extent to which the quenched disorder will affect the quantum phase transitions is determined by the so-called Harris criterion, $d\nu > 2$, where d is the dimensionality of the system (7). Systems that violate the Harris criterion will be susceptible to the effects of quenched disorder. Disorder generally has a substantially stronger effect on quantum phase transitions because the disorder is perfectly correlated in the imaginary time dimension. The rare regions in quantum phase transitions are finite in space but infinite in imaginary time, which enhances the Griffiths phenomena (see the figure) (2).

This enhancement of the effects of the rare regions was first discovered in a 2D classical Ising model that consists of interacting spins on a lattice (8, 9), and its signatures have been reported in 3D magnetic systems (10), but it has not been observed in 2D superconducting systems before. Investigating the quantum phase transition between a superconducting and a weakly insulating or metallic state in ultrathin gallium films, Xing *et al.* find experimental evidence of a diverging dynamical critical exponent near the quantum phase transition.

The observation of the quantum Griffiths singularity in a 2D superconductor offers a new perspective on the previous studies of the superconductor-insulator and metal-insulator transitions. Although these transitions have been studied for decades, there are lingering unexplained inconsistencies between the critical exponents found in different physical systems, lack of universal behavior, and substantial uncertainty in locating the quantum regime. Some of these issues can now be possibly attributed to the effects of quenched disorder, thus recovering the elegance of being able to treat different systems within the same theoretical framework. ■

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The effect of rare ordered regions on the phase diagram.

(A) The phase diagram of a two-dimensional superconductor as a function of temperature T and magnetic field B . The ordered superconducting phase is shown in blue; the disordered insulating or metallic phase is shown in white. Yellow highlights the regime where the rare ordered regions dominate the transition, causing the scaling exponents to increase to infinity. (B) A schematic example of a rare ordered region in a model system of a 2D lattice of spins.

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DEVELOPMENTAL NEUROSCIENCE

Neurotransmitter-tailored dendritic trees

GABA is both transmitter and morphogen, stimulating neuronal branching in the brain

By Nicholas C. Spitzer

GABA (γ -aminobutyric acid) is the major inhibitory neurotransmitter in the mature brain but functions as an excitatory transmitter in the developing nervous system (1, 2). GABA has also been identified as a trophic factor stimulating growth of the embryonic nervous system (3, 4). On page 554 of this issue, Chen and Kriegstein (5) forge a link between neuronal excitation by GABA, calcium signaling, and the morphogenesis of specific neocortical neurons in the growing brain, setting a new standard for analysis of activity-dependent neuronal circuit assembly. The changes in dendritic trees that they report are consistent with those observed in schizophrenia and autism (6, 7).

We are now familiar with multiple functions for single proteins; we should expect no less for single neurotransmitters. GABA is a multipurpose, four-carbon nonprotein amino acid generated by glutamic acid decarboxylase or alcohol dehydrogenase. It regulates quorum sensing and enhances virulence in bacteria, buffers oxidative stress and inhibits forms of autophagy in

yeast, and is important for stress resistance in plants.

It has been a puzzle to understand how neurotransmitters, known to play a key role in transmission of information on the scale of milliseconds, influence the differentiation circuits of neurons on the scale of hours to days. Previous studies that blocked transmitter synthesis or transmitter receptors over broad regions of the nervous system have not pinpointed GABA's mechanism of action in neuronal development.

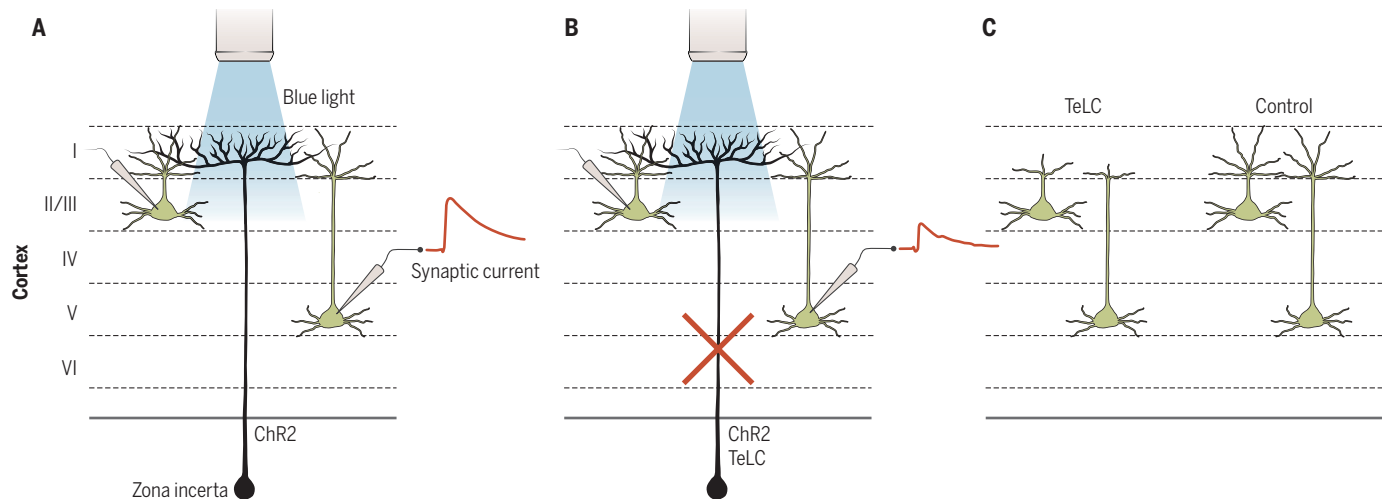
Neuroscientists have recently recognized the central importance of understanding the basis of assembly and function of individual neuronal circuits as a key to understanding how the brain works (8). Chen and Kriegstein have chosen a precision dissection approach to investigate a single neuronal microcircuit. They show that local delivery of GABA to target neurons at an early postnatal stage of development is specifically required for appropriate differentiation of their dendritic trees.

Axons from the zona incerta (ZI), a cluster of neurons deep in the brain, project widely throughout the brain and spinal cord. Chen and Kriegstein found that the axon terminals branch extensively in a plexus, or network, at the surface of the newborn mouse somatosensory and motor neocortex. There they make excitatory synaptic connections with pyramidal neurons located in both

deep and superficial layers of the cortex that extend apical dendritic branches into the plexus (see the figure, panel A). Optogenetic stimulation of ZI neurons elicited depolarizing synaptic currents in these neurons that were blocked specifically by GABA receptor antagonists. Selective viral expression of tetanus toxin in ZI neurons on one side of the animals to block transmitter release chronically after birth suppressed this excitatory GABAergic synaptic input unilaterally (see the figure, panel B). By the end of the first postnatal week, the number of apical dendritic branches of both superficial and deep neurons, which would normally be excited by ZI activity, was reduced (see the figure, panel C), and the number of dendritic spines on which synapses were made was decreased compared to the contralateral control.

Similar circuitry was identified in the human brain, which has extensive morphological GABAergic synapses in the superficial layer of the cortex as early as the end of the first trimester of pregnancy. Dendritic arbors of neurons in deep but not superficial layers of human cortex were found to receive functional GABAergic synapses in the late second trimester. Imaging of intracellular calcium revealed that GABA drove increases in calcium concentration (5) as in mice (9, 10), confirming that GABA is excitatory and suggesting a similar morpho-

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Tailoring dendritic trees (A) Illumination of a rhodopsin-linked membrane channel activated by blue light [channelrhodopsin-2 (ChR2)] in ZI axon terminals in slices of postnatal mouse cortex. Channel activation drives GABA release, eliciting depolarizing synaptic currents (red trace) in deep and superficial cortical pyramidal neurons. **(B)** Coexpressing tetanus toxin light chain (TeLC) to block GABA neurotransmitter release suppresses synaptic currents (red trace) elicited by optogenetic activation. **(C)** Chronic synaptic blockade reduces the number of branches in apical dendritic arbors; basal dendritic branching is not affected.

genetic role for GABA-dependent calcium signaling in neuronal differentiation in the human brain.

To assess the role of this microcircuit in the mature nervous system, Chen and Kriegstein examined its function in mice at 3 weeks of age. GABA is inhibitory in other circuits after the first postnatal week in mice (2, 11). Optogenetic activation of ZI GABAergic neurons generated synaptic currents in deep-layer cortical neurons. These currents suppressed bursting activity induced by removal of magnesium ions that lowers the threshold for excitation. These results demonstrate that ZI neurons are inhibitory at this stage and can suppress high-frequency epileptiform firing.

How do increases in calcium concentration in dendritic trees stimulate growth and branching in the developing nervous system? The excitatory neurotransmitter glutamate, acting via *N*-methyl-D-aspartate (NMDA) receptors, engages Rho guanosine triphosphatases that modulate the actin cytoskeleton (12). Global blockade of GABA receptors or GABA synthesis in the developing retina reduced dendritic branching (13). Eliminating the excitatory action of GABA in a small population of rat ventricular progenitor cells and cortical neurons similarly led to a shorter and smaller number of dendritic branches (14).

Understanding the mechanisms of assembly and function of the nervous system requires analysis at the microcircuit level, like that of ZI input to cortical pyramidal neurons. The effects of GABA on dendritic arbors described by Chen and Kriegstein must act via the cytoskeleton; whether this is at the posttranslational or transcriptional level, or both, as well as the involvement of trophic factors and glia, will be subjects of future work. ■

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VIROLOGY

Visualizing trans-infection

Systemic spread of a virus starts with multiple modes of transmission in the lymph node

By Thomas J. Hope

A fuller understanding of how a virus establishes infection should help in developing approaches that minimize the associated pathology. On page 563 in this issue, Sewald *et al.* (1) succeed in visualizing interactions between retroviruses and cells within the immune tissues of live mice. This is exciting and notable for two reasons. It provides visual insights into the earliest steps leading to systemic infection in a living animal. Additionally, it demonstrates that multiple modes of infection can be used by a virus during dissemination. The observations described begin to reveal the complex steps that a virus must take to establish systemic infection.

Live-animal imaging provides critical insights into biological function and kinetics that cannot be recapitulated within a culture dish or test tube. The physiological and anatomical characteristics of organs and tissues all play a role during infection of a live animal. Intravital imaging (such as multiphoton microscopy) is a technique that has been especially consequential to the understanding of cellular function, localization, and motility of cells within living tissues. Using this approach, a study that focused on the dynamics of HIV-infected cells in humanized mice (2) showed that infection can influence cell mobility within lymph nodes.

Sewald *et al.* have extended that work to include visualization of viruses, allowing bulk particle tracking within the lymphatic vessels of the mouse. Within minutes of a high-dose injection of mouse leukemia virus (MLV) particles into the footpad of mice, the virions are transported to the draining popliteal lymph node by lymphatic flow. Imaging reveals the capture of virus by macrophages lining the subcapsular sinus of the lymph node as they filter debris from the lymphatic system—that is, the macrophages engulf cellular fragments, microbes, and foreign matter. The macrophage-virus interaction is mediated by the cell surface protein CD169, which binds to sialic acid in the viral membrane of MLV and hu-

man immunodeficiency virus (HIV). Once a macrophage has ingested a virion, it can then present viral peptides to immune cells, stimulating immune responses to intact antigenic determinants (3). However, Sewald *et al.* show that MLV exploits this filtering system to its own advantage.

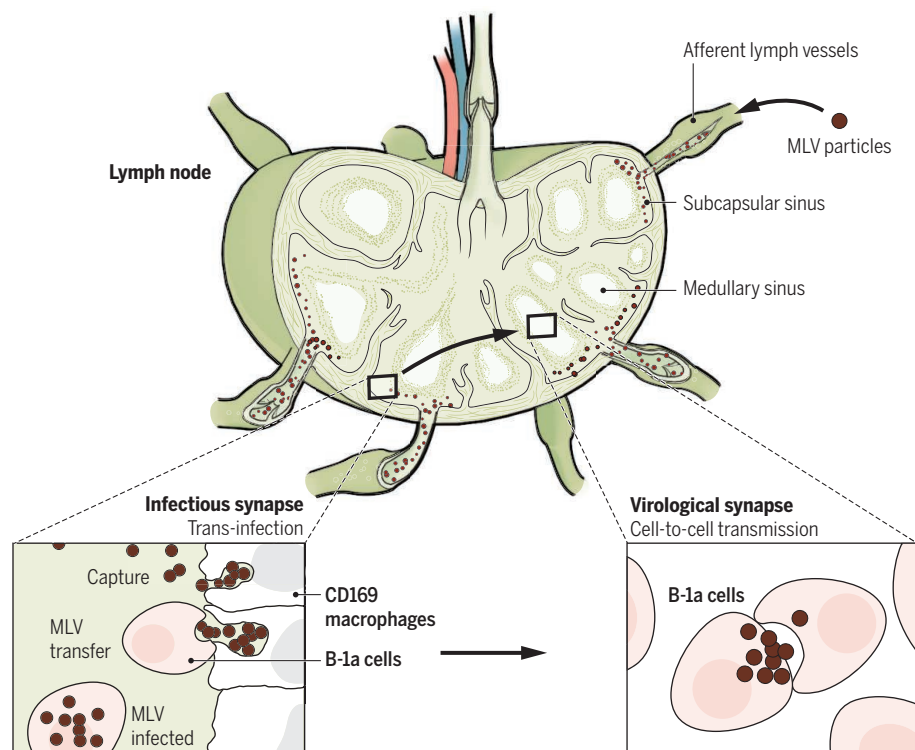
Like macrophages, dendritic cells are also antigen-presenting cells. They sample their surroundings for pathogens and present antigenic determinants to T cells to stimulate immune responses. Interestingly, dendritic cells were found to facilitate the HIV infection of T cells within the same cell culture (4). The finding that the dendritic cell surface protein DC-SIGN can bind to the HIV envelope without triggering cell-virus fusion led to the model of trans-infection (5). In this concept, a cell “holds” a pathogen without getting infected (the pathogen does not enter the cell), only to pass it to another cell to infect it. The mechanism of trans-infection was revealed by imaging

“...evolutionary pressure... turns viruses into masters at leveraging normal biological function to enhance infection.”

fluorescent HIV in live and fixed primary cell cultures (6). A dendritic cell presents an HIV particle to the target T cell while the target T cell concentrates HIV receptors and co-receptors at the site of contact, forming an infectious synapse.

Similarly, Sewald *et al.* demonstrate that MLV particles are “captured” by macrophages and sequestered within folds of the membrane. The virions then can be passed on to a rare set of B cells (B-1a cells) that are the ultimate targets of MLV. This is a clear in vivo validation of trans-infection. In a real infection, where the amounts of virus are much smaller (potentially a single virion), trans-infection becomes much more important: A macrophage can capture the single virion and greatly increase the odds that it can be successfully presented to a rare B-1a cell to initiate the infectious cas-

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Dissemination. MLV particles enter the lymph node, where they are captured by macrophages lining the subcapsular sinus. (**Left inset**) Rare B-1a cells contact the macrophages and are infected with the virus through infectious synapses (trans-infection). (**Right inset**) Infected B-1a cells migrate into the lymph node, spreading infection to other B-1a cells through virological synapses.

cade. This model is corroborated because when CD169 expression on macrophages is decreased either through the use of transgenic mice lacking CD169 or injection of antibodies to CD169, the amounts of infection are greatly decreased. The authors show similar results with fluorescently labeled HIV in a humanized mouse model, revealing evidence of trans-infection with HIV through the same mechanism. This indicates that a comparable interaction can take place with another virus.

In the context of a tissue culture dish, infection by cell-cell transmission of a retrovirus between an infected cell and a target cell, known as a virological synapse, is more efficient than infection by free-floating viral particles (7). In this case, new viral particles are assembled at the site of contact. Sewald *et al.* visualized virological synapses as the viral proteins polarized to the site of contact between infected and uninfected B-1a cells. These observations demonstrate that MLV can use virological synapses to spread among immune cells in the lymph node. Therefore, it appears that MLV uses both infectious synapses and virological synapses to expand the populations of infected cells in a lymph node while establishing systemic infection (see the figure).

Extending these observations of MLV in its natural host to what happens during HIV

infection is a worthwhile pursuit. The data presented by Sewald *et al.* are consistent with trans-infection of HIV in the humanized mouse, but the identification of HIV virological synapses *in vivo* remains elusive. Perhaps the propensity of HIV-infected T cells to fuse with uninfected T cells to form syncytia, which is likely not advantageous to the virus, discourages HIV from using this mode of transmission *in vivo*.

The evolutionary pressure on viruses to be maximally efficient by taking advantage of the host's anatomy and cellular environment turns viruses into masters at leveraging normal biological function to enhance infection. Future intravital imaging studies should reveal new secrets of the viral life cycle and of the progression from initial infectious events to systemic pathology. Uncovering secrets of the HIV life cycle should help to identify weak points that can be pharmacologically targeted to attack the virus and treat or prevent infection. ■

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ECONOMICS

From the lab to the real world

Laboratory experiments provide precise quantitative predictions of peer effects in the field

By Gary Charness and Ernst Fehr

Until the late 1980s, textbooks portrayed economics as a nonexperimental science because it was thought that “Economists...cannot perform the controlled experiments of chemists or biologists....Like astronomers or meteorologists, they generally must be content largely to observe” (1). Since then, economics has experienced an experimental revolution (2–6). However, there has been a debate on the extent to which insights from economic lab experiments can be generalized to field settings (7–11). On page 545 of this issue, Herbst and Mas (12) show that the results of a class of lab experiments can be generalized to the field because they provide quantitatively precise descriptions of productivity spillovers between workers.

The question of how an increase in a worker's productivity affects a co-worker's productivity—termed “productivity spillover”—has implications for wage setting, economic growth, work team composition, workplace organization, incentive structure within organizations, and social returns to human capital. Herbst and Mas collected data from lab and field studies investigating this effect, conducted on several continents. They show that laboratory estimates of the effect are quantitatively very similar to those in field studies. The between-study variance in the quantitative estimates is also very similar in the lab and the field. The analysis thus indicates that results in lab experiments can be generalized to the field.

Generalizability is a concern not only between lab and field studies, but also among field studies. For example, a particular intervention in a field experiment may improve educational outcomes in remote Tanzanian villages, but the same field experiment may fail to improve outcomes in, say, India. A minimum-wage increase in France



may reduce the employment of low-skilled French workers, whereas a similar wage increase in New Jersey may leave employment unaffected. Thus, field-field generalizability poses problems similar to lab-field generalizability because institutional and other contextual details may differ across settings.

The debate on lab-field generalizability (7–9) has focused on the following arguments: The participants may behave differently because they feel observed; they may not be representative of the overall population; the stakes are relatively low compared to real-world situations; and the experimenter may not be able to bring the field context into the lab.

In principle, all these issues can be solved (8, 9). Subjects' behavior is largely unaffected by whether they are observed by an experimenter (10). Moreover, being observed is not exclusive to the laboratory. Many decisions in the field are observed by, for example, anonymous bystanders, colleagues, or friends. Observers can be added to the experimental protocol, allowing the relevance of observability to be assessed by varying the degree of anonymity.

Laboratory experiments often make use of students, who may or may not be representative of the general population or a target population of interest. Even so, many lab

experiments have been conducted with non-student samples such as soldiers, Chinese central planners, professional arbitrators, professional financial traders, manufacturing workers, and subject pools representative of whole countries, with results similar to those with students.

Regarding the issue of small stakes, subjects do appear to be highly motivated by modest laboratory stakes and usually pay attention in experiments. Lab experiments have also been conducted in locations where the stakes translated into several months' earnings, with evidence that average behavior was quite similar to behavior in treatments with lower stakes (13). Furthermore, many decisions people make on a daily basis do not involve large stakes, implying that behavior in small-stakes experiments may be generalizable to these situations.

Finally, fine details of the decision context matter, such as the framing of a task. But carefully conducted laboratory studies offer far better controls of contextual factors relative to the field. Therefore, it may be possible to understand the behavioral effects of contextual cues that prevail in the field by varying these cues in suitable ways in the lab.

As Herbst and Mas show, suitably designed lab experiments can provide useful insights about the behavior of people outside the lab. But few experimental economists would have expected lab results to capture the quantitative effects that prevail in the field. In fact, some have argued that

lab experiments should not even aim at this goal (11). Thus, even those who are convinced that lab experiments in economics are a powerful method for understanding important aspects of the real world will be surprised and intrigued by the results reported by Herbst and Mas. The study may even change some scientists' views of the relevance of lab experiments. But because it is not possible to draw general conclusions from a single study, we hope to see more such valuable studies in the future. ■

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IMMUNOLOGY

Synthetic immunobiology boosts the IQ of T cells

Engineered chimeric antigen receptors show promise

By Michael C. Jensen

Pharmaceutical small-molecule drugs are the first “pillar” of modern medicinal therapeutics, with recombinant protein biologics claiming the second pillar. If the emergent third pillar of medicine is cell-based therapeutics, cellular immunotherapy of cancer stands as the pillar’s current poster child (1). This approach includes adoptive T cell therapy, which has seen major advances recently. Underlying some of this progress are developments in synthetic tumor recognition receptors. Although it’s early days for applied synthetic immunobiology, increasing momentum in this field may soon lead to the application of engineered T cells to a broader spectrum of cancers as well as to infectious and autoimmune diseases.

The ability to engineer a patient’s T cells to express synthetic chimeric antigen receptors (CARs) that recognize specific tumor antigens (2) has advanced adoptive T cell therapy. However, dramatic clinical responses can be accompanied by “cytokine storm” and neurologic toxicities. This is due, in part, to synchronous and sustained activation of CAR T cells by large quantities of tumor cells. CAR T cells are, in essence, an uncontrolled runaway therapeutic because of their fixed functional “ON” status, a consequence of vector systems that use constitutive promoters to express these receptors.

Although cellular suicide mechanisms can be engineered into engrafted T cells to allow for their inducible termination, such an intervention can prematurely attenuate therapeutic potential (3). There has been interest in developing CAR T cells that are equipped with an “ON-OFF” switch that is controlled by clinician-prescribed small-molecule inputs, and Wu *et al.* (4) have made a major step. The authors disassembled a CAR into two parts by expressing the extracellular antigen-binding domain separately from the intracellular signal-transducing domain (which mediates T cell activation). By reassembling a bipartite CAR in human T cells in the presence of a drug, Wu *et al.* produced CAR-redirectioned

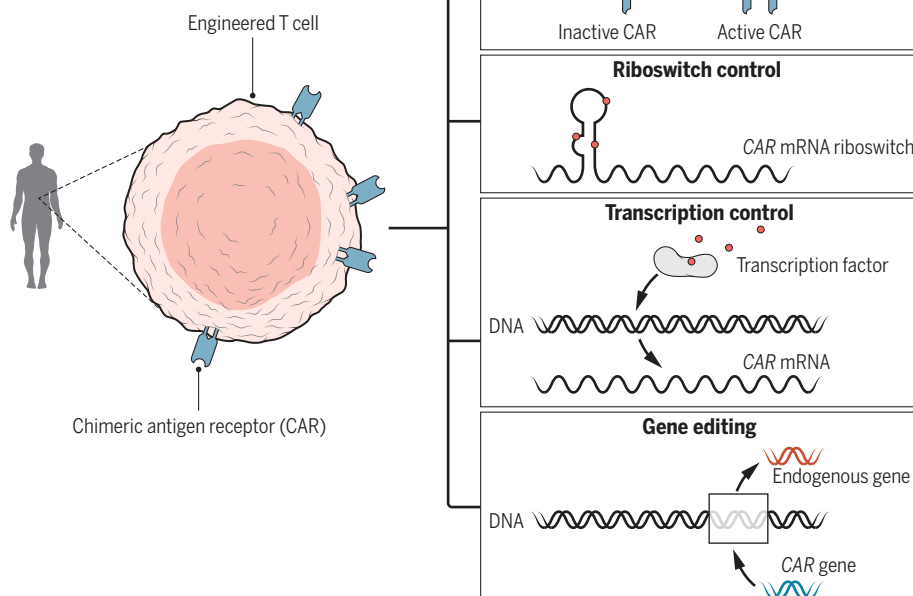
T cell antitumor function that was rendered drug-dependent (see the figure).

The design principles used in developing a regulated ON-switch CAR reflect a growing alliance between the disciplines of synthetic biology and cellular therapeutics development. The workspace for engineering encompasses a growing number of drug-regulated transcriptional control systems, messenger RNA (mRNA) riboswitch devices, and posttranslational protein stability switches (5–7). Many of the initial proof-of-concept devices (such as chimeric transcription factors) were derived from xenogeneic parts that had prohibitive immunogenicity profiles and were regulated by small molecules with unfavorable pharmacodynamics and off-target toxicities. Today’s next-generation systems use human components and drugs with favorable attributes for the clinic, including small-molecule designer drugs that are otherwise bioinert (8). This toolbox of devices affords opportunities to assemble logic-gated control systems that can integrate an input (such as a clinician-prescribed drug), and

to further refine where, when, and to what extent a CAR T cell deploys its functions.

In parallel with the development of synthetic control systems, a revolution in gene editing—exemplified by the clustered regularly interspaced short palindromic repeats (CRISPR) and transcription activator-like effector nuclease (TALEN) platforms—has captured the imagination of the CAR T cell engineering field, particularly as it relates to removing key immunoregulatory genes [such as programmed cell death protein 1 (PD-1), a so-called checkpoint inhibitor] that restrain T cell function (9). Conceptually, releasing checkpoint inhibition by gene editing of CAR T cells may be safer than systemically blocking checkpoint inhibition with pharmaceuticals. Moreover, the high efficiencies of these gene-editing platforms suggest that the simultaneous removal of multiple immunoregulatory genes in individual T cells for additive or synergistic effects is now feasible. Thus, specific nucleases can be used to edit out endogenous genes in a T cell, followed by the swapping in of a modified or different gene through homologous recombination. Indeed, Sather *et al.* introduced meganucleases and replacement DNA templates (through mRNA electrotransfer and adeno-associated virus vector transfection, respectively) into primary human T cells at startlingly high frequencies of homologous recombination (10). In this case, the gene encoding C-C chemokine receptor type 5 (the receptor for HIV) was edited out of human T cells and replaced with DNA encoding a

T cell engineering. Future immunotherapy may include engineering a patient’s T cells to express chimeric antigen receptors. Possible strategies include those controlled by a clinician-prescribed small molecule or by gene editing.



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CAR with HIV-specific reactivity. The resulting CAR T cells were rendered resistant to HIV infection and killed HIV-infected target cells in vitro. The advantages of implanting orthogonal therapeutic transgenes into endogenous loci with retained expression regulation of the original gene has far-reaching implications for CAR T cell engineering, as well as for cellular therapeutics in general.

Major challenges persist for engineering therapeutic cells and for next-generation CAR T cell development in particular. These include generic issues related to vector capacity for the large genetic payloads of these regulated systems. Also at issue is the tuning of stringency to achieve functional OFF and the amplitude of induction to achieve an ON-state for the various classes of transgenes (CARs, dominant negative and constitutively active effector proteins, and secreted payloads). Additionally, drug-modulated transgene regulatory systems have largely evolved in the context of a small number of pharmaceutical drugs, and are otherwise bottlenecked by limited types of drug-interacting protein modules that can mediate regulated outputs through dimerization, sequestration, or degradation. A developmental pathway that has not been sufficiently embraced to date is one wherein medicinal chemists co-evolve small-molecule drugs to work in the context of new protein- and RNA-based regulatory platforms. Another major challenge in the clinical use of therapeutic T cells is, ironically, overcoming the immune system's capacity to reject engineered cells based on the immunogenicity of expressed transgenes (17).

As T cell engineering strategies evolve to overcome counterregulatory mechanisms exploited by solid tumors and chronic infections, stringent real-time regulation will be necessary to balance gains in therapeutic potency with patient safety. Side-effect profiles that are acceptable in phase I clinical trials involving patients with poor prognoses will be less palatable to clinicians treating patients earlier after diagnosis, who have numerous therapeutic options. In software development parlance, the field is just transitioning from version 1.0 to version 2.0 CAR T cells, with further iterations to come. ■

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EVOLUTION

A window into ape evolution

A fossil of an ape ancestor helps to explain gibbon evolution

By **Brenda R. Benefit and Monte L. McCrossin**

Humans, Old World monkeys, gibbons, and the great apes of Africa and Asia are the only survivors of a highly diverse evolutionary radiation that began at least 28 million years ago (1, 2). The fossil record of human evolution after we diverged from apes is rich, but much less is known about the evolutionary history of modern apes. Not only is the fossil record incomplete but also the morphology of primitive apes from the Miocene (25 to 5 million years ago) seldom conforms to expectations based on living species. The ancestors of gibbons are particularly elusive. On page 528 of this issue, Alba *et al.* describe a Miocene fossil from Catalonia, Spain, that may bridge the gap between earlier small-bodied African apeline primates and living gibbons (3).

During the Miocene, the ancestors of today's Old World monkeys and apes lived alongside other primitive primates that have no living descendants. One such group, the small-bodied pliopithecids (best known from the skeleton of *Pliopithecus vindobonensis*) lived in Europe and Asia 17 to 10 million years ago. Although their faces are gibbon-like, pliopithecids lack some of the derived features shared by Old World monkeys and apes. For example, pliopithecids had a wide ringlike external ear opening, which resembles that of New World monkeys and is unlike the constricted bony ear tube of Old World monkeys and apes (4). Their elbows also retain a hole for the passage of nerves to the inside of the joint (termed entepicondylar foramen) that is not found in Old World monkeys and apes (the reason we feel pain when we bump our elbows).

The ecological counterparts of pliopithecids in Africa between 20 and 14 million years ago were a diverse group commonly referred to as "small-bodied apes" because of their gibbon-like teeth. Unfortunately, their bones did not preserve as well as those of pliopithecids, and only fragments of their skeletons and faces are known. Debate exists over which specimens belong to which species and whether some are related to apes and others to pliopithecids (5, 6). Most lack the hole above the elbow and thus resemble

modern Old World monkeys and apes more closely than do pliopithecids. Without evidence of their ear morphology, it remains unclear whether they were related to living apes or left no descendants.

Alba *et al.* discovered the new species, *Pliobates cataloniae*, in 11.6-million-year-old deposits at Hostalets de Pierola in the Valles-Penedes Basin. It differs from pliopithecids in lacking the hole above the elbow joint and is more like the African small-bodied apes. It is thus the first evidence of the small-bodied ape

"The evolutionary history of Old World monkeys, primitive apeline primates, and true apes reflects the random nature of evolution, with diverse species combining traits in different ways as they adapt to an evolving landscape."

radiation in Europe. Furthermore, *Pliobates cataloniae* is represented by an associated and well-preserved partial skull and skeleton. The skeleton of *Pliobates* is therefore an important touchstone for understanding the relationships and adaptations of these small apeline primates.

Alba *et al.* place *Pliobates cataloniae* into a new family of apes, the Pliobatidae. As for many African Miocene apes, including the primordial larger-bodied ape *Proconsul*, *Pliobates* was tree-dwelling, with limbs designed for walking with palms down along the tops of branches. However, several aspects of the wrist of *Pliobates* are more similar to modern apes than are those of *Proconsul*. These features allowed the *Pliobates* wrist to rotate during cautious climbing and clambering. The *Pliobates* elbow, however, lacks a critical feature that differentiates living apes from other primates: the spool-shaped raised and well demarcated keel (lateral trochlear keel) that allows living apes to stabilize their elbow joints while hanging by their arms.

The skull of *Pliobates* combines a confusing mosaic of primitive and derived features. Alba *et al.* used three-dimensional

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imaging techniques to digitally isolate cranial fragments, and fill in missing pieces by mirror-imaging those that are preserved to reconstruct the skull as accurately as possible. The broad forehead and rounded neurocranium indicate a brain volume of about 70 cm³ for *Pliobates* (3), twice as large relative to its body mass as the similarly sized primitive Miocene Old World monkey *Victoriapithecus* (7). *Pliobates*' brain size is similar to that reached only very recently (during the Holocene) in modern Old World monkey. *Pliobates* shares some facial characteristics with living gibbons, including goggle-like orbital rims, which hints at its potential ancestry to gibbons (3). At the same time, the external bony ear of *Pliobates* is more primitive than that of the 28 million-year-old ancestor to the Old World monkey and ape clade *Saadanius* (1), the 18-million-year-old ape *Proconsul*, the 15-million-year-old Old World monkey *Victoriapithecus*, and all modern Old World monkeys and apes (3). The ear of *Pliobates* has a wide external opening that is strikingly similar to *Pliopithecus* and New World monkeys and is difficult to reconcile with its other apelike features.

The combination of primitive and derived traits in *Pliobates* makes it certain that scientists will debate its placement in the newly erected ape family Pliobatidae (see the figure). Alba *et al.*'s interpretation of *Pliobates* as a small-bodied ape rather than a pliopithecid requires independent evolution of a constricted bony ear tube in Old World monkeys and apes. Since the

molecular clock suggests that gibbons and great apes split around 14 million years ago, another possibility is that *Pliobates* is ancestral only to gibbons, which would mean that both the bony ear tube and the sharp lateral trochlear keel of the elbow evolved independently in gibbons and great apes. Alternatively, *Pliobates* may be a pliopithecid in which specializations of the elbow and wrist similar to those of living apes emerged through convergent evolution. Whichever interpretation is true, convergent evolution must have occurred in ape evolution, given the combination of primitive and derived traits in *Pliobates*.

Mosaic combinations of primitive and derived traits are not unique to *Pliobates* among the Miocene Old World monkeys and apes. For example, several lineages of Miocene apes (including *Sivapithecus* from Pakistan and *Kenyapithecus* from Kenya) had monkey-like shoulder joints different from those in living apes (8–10). Yet, other features of these fossils, such as dental, facial, limb, and wrist morphology, resemble those of living apes (11). Larson (12) has argued for independent and parallel evolution of the ape shoulder characteristics in Asian and African apes. If true, this would allow *Sivapithecus* to be ancestral to orangutans and *Kenyapithecus* to African apes. Alba *et al.* follow similar logic when arguing that *Pliobates* is a member of the ape clade rather than a derived pliopithecid despite its primitive ear region.

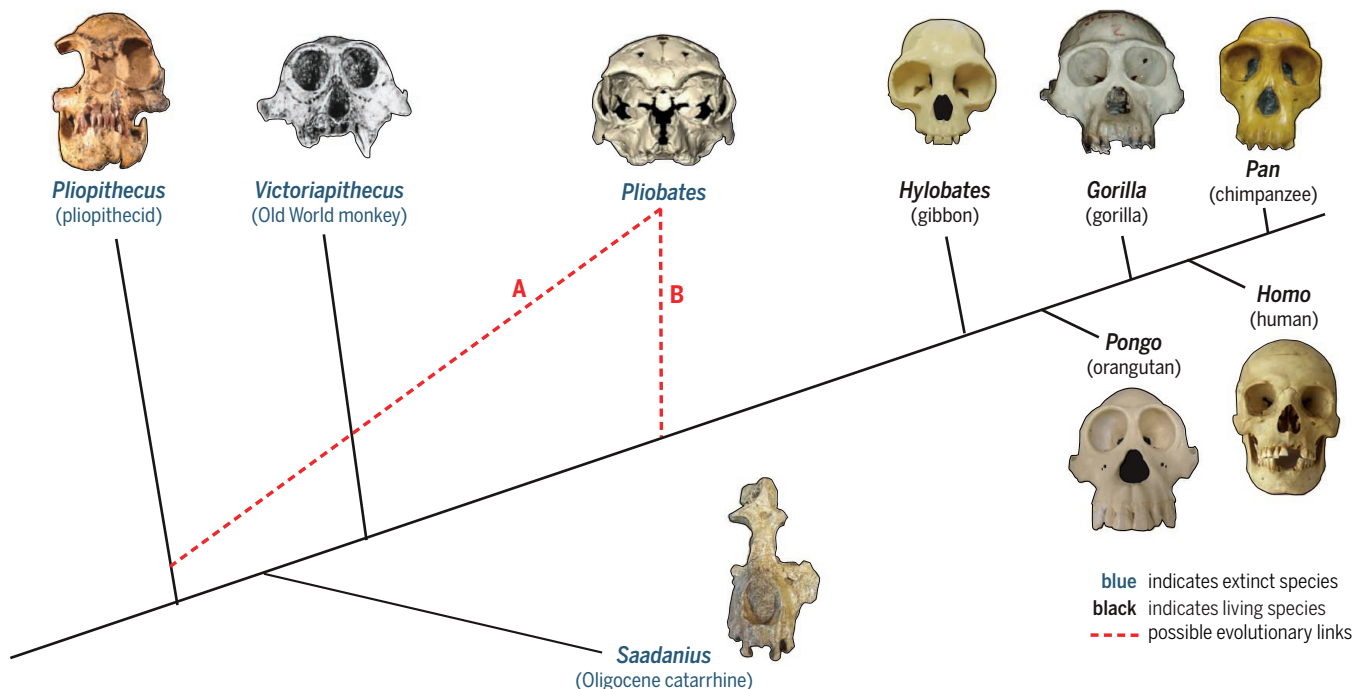
The evolutionary history of Old World monkeys, primitive apelike primates, and

true apes reflects the random nature of evolution, with diverse species combining traits in different ways as they adapt to an evolving landscape. Convergence appears to have played a major role in the emergence of modern apes, but which features evolved in parallel? Cladistic analysis of morphological traits is an imperfect tool that reflects how each scientist breaks up and codes complex morphologies while ignoring potential developmental correlations, intraspecific variation, and missing fossil data. Only the discovery of other complete fossils, like that of *Pliobates*, and a better understanding of the mechanisms influencing morphological development and convergence will help to elucidate the complex history of ape evolution. ■

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Ape evolution. A primitive primate fossil reported by Alba *et al.*, termed *Pliobates*, may either have been a pliopithecid (A) or an ape (B). Skulls are not to scale.

Eric Davidson (1937-2015)

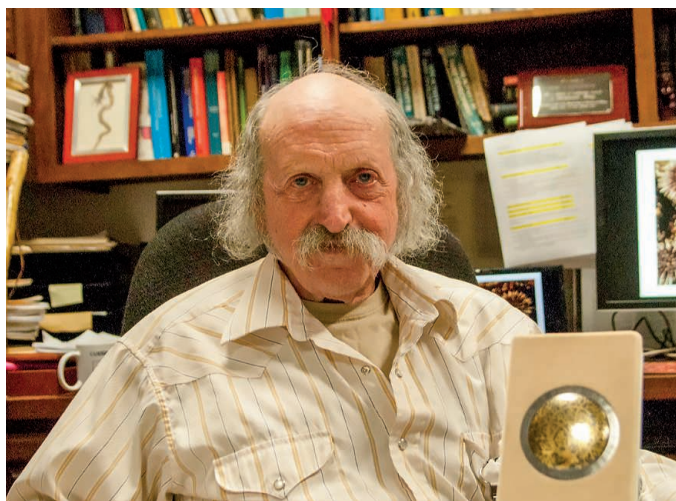
A developmental biologist uncovered the structure and evolution of gene regulatory networks

By Douglas H. Erwin

Eric Davidson made major contributions to elucidating the mechanisms and logical structure of developmental gene regulatory networks. The Norman Chandler Professor of Cell Biology at the California Institute of Technology, Eric died of a heart attack on 1 September 2015 in Pasadena, California. Eric's death came just a few months after the publication of his latest book, *Genomic Control Process: Development and Evolution*, coauthored with his colleague Isabelle Peter. In this book, Eric and Isabelle continued to push forward his career-long project of applying rigorous experimental, modeling, and conceptual studies to understanding mechanisms responsible for the operation of gene regulatory networks, as well as how they had evolved through time.

Parallel interests in development and evolution were evident in some of his earliest work. In 1969 and 1971, Eric and his long-time friend and collaborator, the molecular biologist Roy Britten, published two fundamental papers on gene regulatory networks. In the first paper, they developed a theory of gene regulation and cellular differentiation, and perceptively argued, based on DNA content, that the primary difference between a sponge and a mammal lay not in the number of genes but in "a vastly increased complexity of regulation." When Eric wrote the 1969 paper, he was still at Rockefeller University in New York, where he had studied for his Ph.D. in 1963 under Alfred Mirsky, a pioneer in molecular biology. By 1971, when Roy was still at the Carnegie Institution but Eric had moved from Rockefeller University to the California Institute of Technology (CalTech), they had begun exploring repetitive DNA. Eric and Roy expanded their model of gene regulation and discussed how the evolution of regulatory patterning could generate novel morphologies.

Eric had a deep interest in the fossil record and particularly in the rapid appearance of diverse animal clades near the base of the Cambrian (the period between 542 to 488 million years ago). Eric and I met in 2001 when he asked to stop by the U.S. National Museum of Natural History for a tour of the middle Cambrian Burgess Shale fossils. Eric



was in Washington, DC, to try to convince the National Aeronautics and Space Administration to fund research in evolutionary biology (by which Eric meant the evolution of gene regulatory networks). I devoured these papers in college and followed Eric's expanding program of research on gene regulation. Eric was a bit surprised by the keen interest of a paleobiologist in his work, but he invited me to visit CalTech a few months later, which led to our first paper.

Long collaborations between developmental biologists and paleontologists are infrequent, even anomalous, perhaps. But Eric and I shared a deep interest in what the growing insights from gene regulatory networks would reveal about the early evolution of animal body plans. We slipped into an easy routine (as easy as anything could be with Eric). When I visited CalTech, we would begin with long conversations about the latest work on gene regulatory networks and on the early record of animals. Back and forth, we would eventually narrow in on our next project.

Eric was driven to understand the mechanisms of development at the most fundamen-

tal level possible. His efforts since the late 1960s illustrate the incredible experimental growth in the field, driven in part by technological and methodological advances. But Eric's contributions were just as strong on the conceptual side, aided by his prodigious knowledge of the literature and the successive development of more refined models of gene regulatory networks.

Our collaborative work was based on the experimental and conceptual work of his group and other developmental biologists, as we puzzled out the implications of the data for evolution. Eric's extremely logical mind could not abide either sloppy experimental work or shoddy thinking. Anything that, at least to him, did not advance the search for mechanism (or that differed from his intuition, although he rarely admitted this) would be dismissed with a sweep of his hand and an emphatic statement that something was "pre-scientific rubbish" (Eric actually used an expletive for "rubbish"). This led to not a few of our conflicts. I often included citations to intellectual predecessors, but Eric claimed that his reputation would suffer if he cited anything published more than 5 years previously. This led to an epic battle in 2009 over my efforts to cite a 1978 book by Austrian evolutionary biologist Rupert Riedl, whose work anticipated some of the ideas that Eric and I were advancing.

After a battle lasting several months (yes, months), we compromised with a citation to a recent discussion of Riedl's work. Eric's reputation was preserved.

Over the years of our collaboration, his group developed a progressively more detailed understanding of the architecture of gene regulatory networks in *Strongylocentrotus*, Eric's favorite sea urchin. The introduction of a Boolean logic framework was a major advance, and I still recall his excitement on discovering a "double negative" logic gate within the network. As Eric's group continued to unravel the logical structure of these networks, they revealed new possibilities for understanding their evolution. This past summer we had begun developing some experimental approaches to understanding gene regulatory network divergence between two groups of echinoids (sea urchins) that separated about 250 million years ago. As the future brings new and surprising insights into developmental evolution, the rest of us will have to guard against "prescientific rubbish." ■

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LETTERS

Edited by Jennifer Sills

Hunted carnivores at outsized risk

IN THEIR REPORT “The unique ecology of human predators” (21 August, p. 858), C. T. Darimont *et al.* explain that human predation pressure is considerably higher on large carnivores compared with other faunal groups. Evolutionarily speaking, the high predation pressure exerted by humans is a very recent phenomenon for large carnivores (1, 2). As a result, the impact of human predation is even more severe than the number of removed animals suggests. However, despite increasing evidence, indirect impacts of killing apex predators are still commonly overlooked when managing large carnivores.

Mesocarnivores and herbivores have typically evolved under continuous predation pressure throughout their evolutionary history and have, in response, developed various phenotypical, behavioral, and life-history adaptations to predation (3, 4). On the contrary, because of the apex position of large carnivores in trophic webs (2), this group has not faced the same predation evolutionary pressure and is thus expected to be less adapted to high predation. This may explain the extirpation of large carnivores in many regions of the world when compared with mesocarnivores (2).

Human killing of large carnivores has been associated with several undesirable side effects for surviving members of the mutual social groups or related individuals. Examples include increased levels of infanticide following shooting of breeding male lions (5) and brown bears (6), disrupted dispersal patterns in leopards (7), and increased hybridization or disrupted social structure in wolves (8, 9), with further consequences in human-predator conflicts (10).

If we want to restore healthy, functioning ecosystems and the services they provide, we must implement effective measures such as banning hunting around breeding periods and preventing removal of key members in social groups. Only by integrating such strategies can we mitigate undesirable side effects of hunting.

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OUTSIDE THE TOWER

Repainting citizen science

How do colors affect attention and learning? Could repainting a classroom improve the learning of the students within? These were the questions asked by students of Molins de Rei High School in Catalunya, Spain. After seeing an

online video posted by their class, we—two cognitive neuroscience researchers and one mediator—decided to help them find answers.

Most citizen-science programs are defined by researchers who invite citizens to contribute by collecting data (1). Some programs consider questions formulated by citizens, in cases where those questions are relevant for academic research [e.g., (2)]. But what if researchers involved citizens in the experimental part of the research? Engaging the public in hypothesis creation and testing can be beneficial for both parties: It anchors the research in an ecological setting, while stimulating citizens' curiosity and providing them with tools for critical thinking.

After helping the students refine their question, we taught them how to build a protocol and perform statistical analyses. We worked with them to replicate published data and then to design and perform original experiments in the school (3). Our preliminary results suggest that the influence of colors on learning varies between individuals and depends on baseline distractibility. We are working with the students on follow-up studies, and one of us (L.R.-S.) has initiated similar projects with adults (4).

Taking part in this process was empowering for the students, to the point where they became advocates of participatory research (5). In turn, they challenged our traditional way of doing research, urging us to think out-of-the-box and thus improve as scientists.

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Outside the Tower is an occasional feature highlighting science advocacy projects led by scientists and citizen scientists. How do you advocate for science? Tell us at submit2science.org.

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Policies undermine Brazil's GHG goals

IN A BOLD move, Brazil has submitted to the 21st conference of the parties (COP21) in Paris an intended nationally determined contribution (INDC) to reduce by 2030 its greenhouse gas (GHG) emissions by 43% in relation to 2005. This target goes well beyond other developing countries and is above the pledge of the United States and not far from the proposal from the European Union, despite their greater historical responsibility. However, current policies and actions announced by Brazil are unlikely to be enough to meet the proposed GHG cuts from land-use change.

In order to meet its INDC, Brazil seems to assume that the end of illegal deforestation in the Amazon and the implementation of the market of environmental reserve quotas (CRA) are going to be enough to drastically reduce the country's total emissions from the land sector. But a close analysis of these policies shows otherwise.

Since 2012, farmers who do not have the required amount of Legal Reserve (mandatory private conservation area) can compensate by purchasing CRA offsets—titles to portions of forest located on properties that have more than the required Legal Reserve. In theory, this should limit the total deforestation, but there is a loophole. Depending on pending regulatory choices, the offset market could be flooded with 14 million hectares (Mha) of low-cost titles from private lands inside already protected areas and 38 Mha from Legal Reserves of small properties that are already protected by the Forest Code (1), meaning that no additional forests are saved. This allows farmers with forest debt to purchase cheap offsets while others can legally clear their own land.

Increased forest governance in the Amazon led to a substantial reduction in deforestation (2, 3). However, this biome still has 12 Mha of native forests that could be legally deforested (3) and 39 Mha of undesignated land (4) open for land grabbing and new settlement projects. The situation is particularly worrisome in the Cerrado biome—the most coveted region for agribusiness expansion—where 80% of the private property can be legally deforested. Deforestation in the Cerrado

currently contributes to 26% of emissions from land-use change and is expected to increase because the biome contains 40 Mha (of which 11 Mha are highly suitable for soybeans) that could be legally deforested (3, 5). Therefore, enforcement of the Forest Code unaccompanied by additional conservation policies, such as payment for ecosystem services and protected area expansion, is unlikely to curb emissions from deforestation to the levels promised by Brazil's INDC.

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TECHNICAL COMMENT ABSTRACTS

Comment on “Crystal structures of translocator protein (TSPO) and mutant mimic of a human polymorphism”

Jimin Wang

Li *et al.* (Reports, 30 January, p. 555) reported on a crystal structure for a translocator protein (TSPO) from *Rhodobacter sphaeroides* in which some of the electron density is modeled as a porphyrin. The analysis of the x-ray data discussed here suggests that this assignment is incorrect.

Full text at <http://dx.doi.org/10.1126/science.aab1432>

Response to Comment on “Crystal structures of translocator protein (TSPO) and mutant mimic of a human polymorphism”

Fei Li, Jian Liu, Yi Zheng, R. Michael Garavito, Shelagh Ferguson-Miller

Wang comments that the diffraction data for the structure of the A139T mutant of translocator protein TSPO from *Rhodobacter sphaeroides* should be used to 1.65 instead of 1.8 angstroms and that the density interpreted as porphyrin and monoolein is better fitted as polyethylene glycol. Although different practices of data processing exist, in this case they do not substantially influence the final map. Additional data are presented supporting the fit of a porphyrin and monooleins.

Full text at <http://dx.doi.org/10.1126/science.aab2595>

TECHNICAL COMMENT

PROTEIN STRUCTURE

Comment on “Crystal structures of translocator protein (TSPO) and mutant mimic of a human polymorphism”

Jimin Wang

Li *et al.* (Reports, 30 January, p. 555) reported on a crystal structure for a translocator protein (TSPO) from *Rhodobacter sphaeroides* in which some of the electron density is modeled as a porphyrin. The analysis of the x-ray data discussed here suggests that this assignment is incorrect.

Translocator proteins are implicated in human diseases caused by malfunction of the systems that transport porphyrins, cholesterol, and proteins into mitochondria (1). In addition, *Bacillus cereus* translocator protein (TSPO) and other bacterial TSPOs catalyze a light-dependent photooxidation of protoporphyrin IX (PpIX) (2, 3). Thus, a crystal structure of TSPO with a porphyrin bound is likely to shed light on functional properties of this class of proteins. The structure reported by Li *et al.* describes a TSPO that appears to have a copurified porphyrin bound, which is identified as PpIX in the corresponding Protein Data Bank (PDB) file (PDB accession 4UC1) (4). If this assignment is correct, this structure raises a new set of questions. Because the biochemical data provided by Li *et al.* (4) show that their

purified *Rhodobacter sphaeroides* TSPO (*R*sTSPO) protein still binds externally supplied PpIX tightly, does *R*sTSPO have multiple porphyrin binding sites, and if so, how is the observed site related to the function of the TSPO proteins? Before making the considerable effort it may take to answer these biochemical questions, it might be wise to examine the reliability of the claim that this structure includes PpIX or some other closely related compound.

Close examination of the 4UC1 structure raises several issues, some of which are evident in the figures included in the paper reporting on this structure (4). The PpIX structure shown in figure 4 of that paper appears to be bent instead of planar, and many of its ring substituents do not appear to fit into the “feature-enhanced map” included in their paper. It is also relevant that the PDB validation report for 4UC1 flagged the PpIX and nearly all the headless monooleins that structure is reported to include as outliers both in electron density fitting and in geometry (4). The ligand library density-fitting (LLDF) score

is 11.24 for the PpIX, and it is as high as 30.71 for the monooleins (LLDFs greater than 2.0 are considered a concern). In addition, the geometry outlier *Z* score for the PpIX is 3.53 for bond-length violations, involving 50% of all its atoms, and it is 2.63 for bond-angle violations, involving 40% all of its atoms (*Z* scores greater than 2.0 are a cause for concern). Thus, it appeared that an independent analysis of this structure that takes advantage of the data deposited for 4UC1 might be warranted.

That analysis raised some questions about the structure factors deposited for this structure in the PDB that can only be addressed if the original raw data were publicly available, which they are not. The protein used to obtain the 4UC1 structure contained Se-Met, and the data were collected at the Se peak wavelength. The presence of Se-Met in the protein can be demonstrated by refining the structure assuming that only S-Met is present. Large positive residual peaks appear on the S atoms of S-Met residues in the corresponding difference maps, as expected, and an estimated Se-Met/S-Met substitution ratio is about 76% to 24%. For a typical Se-Met data set that extends to such high resolution, one expects that (i) anomalous difference Fourier maps should include positive peaks that are much larger than the noise for all the Se atoms; (ii) the ratio of the anomalous signals to measurement errors should always be greater than 1—i.e., the differences between the Friedel mates, $F(hkl)$ and $F(\bar{h}\bar{k}\bar{l})$, of Bragg reflections should be larger than their estimated errors; and (iii) an outstanding experimental map should be obtained using Se anomalous signals (5). Unfortunately, none of these expectations is satisfied by the 4UC1 data set. In fact, the reported, average anomalous signal ratio of the 4UC1 data set was smaller than 1, suggesting that the measurement errors might have been systematically overestimated. If this is so, it would also have an adverse effect on the resolution reported for the 4UC1 data set. A better, and possibly higher,

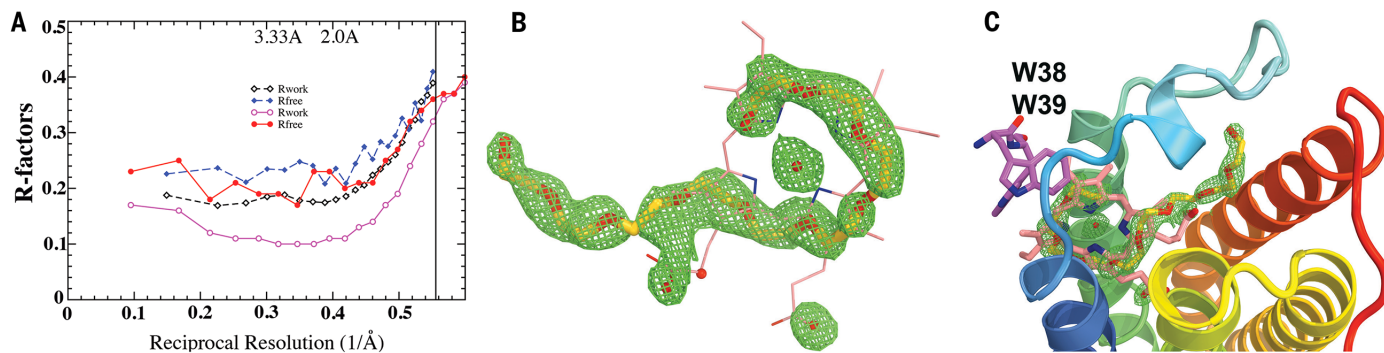


Fig. 1. Structure re-refinement for 4UC1. (A) Fractional R_{work} (magenta open circles) and R_{free} (red filled circles) values after structure re-refinement, using all the available data deposited in the PDB at a resolution of 1.65 Å as a function of reciprocal resolution (1/Å). The reported R_{work} (black open diamonds) and R_{free} (blue filled diamonds) values for the 4UC1 structure are included for comparison at the truncated 1.80-Å resolution marked by the vertical line. (B) Final weighted $2F_{\text{obs}} - F_{\text{calc}}$ map contoured at 1.0 σ is overlaid with a

(PpIX) model (thin salmon sticks) and a PEG molecule (thick gold/yellow sticks). Currently, the density is fitted with 9 PEG units. It can also fit 10 units after converting the centrally bound water molecule into a part of the added unit. The PpIX molecule does not fit the density at all. (C) The presence of two Trp side chains (W38/W39, magenta) may have defined the unusually curled conformation of the PEG. The orientation of this figure is very close to figure 4A of (4).

resolution structure would probably emerge if the data were properly reprocessed.

Although the deposited 4UC1 data set extends to a resolution of 1.65 Å, none of the data between 1.80 and 1.65 Å appear to have been used to obtain the 4UC1 structure or to compute its *R*-factor statistics (4). Exclusion of the weak-intensity high-resolution data, which are invariably poorly explained by structural models, is bound to improve the *R*-factor statistics of a structure, even though it compromises the utility of *R* factors as indicators of structure quality and may make modeling errors hard to detect (6). In fact, when all the 4UC1 data were used for structure refinement and the model corrected by hand, *R* factors improved in all resolution ranges (Fig. 1A). Even for the data between 1.80-Å and 1.65-Å resolution, *R* factors are still below the 42% limit of Evans and Murshudov (7). Furthermore, it was found that electron densities for the putative PpIX

site and several headless monoolein sites were better modeled by other moieties such as low-molecular weight polyethylene glycol (PEG) molecules, the presence of which might be explained by the fact that the crystals in question were grown in the presence of 32% PEG 400 (Fig. 1, B and C). These reassignments of electron density are supported by LLDF values and *Z*-score values that are all below those deemed acceptable.

Of course, there is more than one way to process diffraction data. One may trade radiation-damaged data with redundancy, anomalous signals with resolution, the precision with the accuracy of the data, and so on. Given this fact, discussion is currently under way about whether deposition of raw data should be required for publication of crystal structures so that they can be reprocessed later by others when questions like those addressed above arise in the future (8).

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TECHNICAL RESPONSE

PROTEIN STRUCTURE

Response to Comment on “Crystal structures of translocator protein (TSPO) and mutant mimic of a human polymorphism”

Fei Li, Jian Liu, Yi Zheng,* R. Michael Garavito, Shelagh Ferguson-Miller†

Wang comments that the diffraction data for the structure of the A139T mutant of translocator protein TSPO from *Rhodobacter sphaeroides* should be used to 1.65 instead of 1.8 angstroms and that the density interpreted as porphyrin and monoolein is better fitted as polyethylene glycol. Although different practices of data processing exist, in this case they do not substantially influence the final map. Additional data are presented supporting the fit of a porphyrin and monooleins.

We appreciate Wang's Comment (1) on our structure of the A139T mutant of translocator protein TSPO from *Rhodobacter sphaeroides* (RsTSPO) [Protein Data Bank (PDB) accession 4UC1] (2) and the opportunity to reemphasize the basis for our interpretation of the electron density. Wang claims that a better structure of RsTSPO-A139T could be obtained if 1.65 Å, instead of 1.8 Å, were used as the high-resolution cut-off for the diffraction data and if Se, instead of S, were used throughout the refinement. His Comment also questions the interpretation of portions of the electron density as monoolein molecules, despite the fact that this membrane protein structure was obtained within a lipidic cubic phase (LCP) environment with 60% monoolein. Wang argues that reanalysis of our data shows that polyethylene glycol (PEG) fits the electron density better for both the monooleins and the ring-shaped density that we interpret as a porphyrin-like molecule. Although we welcome reanalyses of our data, we disagree strongly with several key points raised by Wang.

Different practices of data processing and refinement exist, but they rarely substantially influence the final map obtained. In our case, the decision to treat Se as S during refinement was based on the following: (i) the phase for this structure was solved by multiple isomorphous replacement with anomalous scattering with Hg and (Ta₆Br₁₂)Br₂ derivatives before obtaining the 1.8-Å data set and (ii) the data set was not collected at the Se absorption peak (noted in PDB) and does not reliably result in extra density on the S atoms. Because no additional information would be gained by refining the structure as an anomalous data

set, all methionine was treated as S-containing. In fact, we obtained a structure of RsTSPO-A139T at 2.4-Å resolution with an unlabeled protein (PDB 5DUO). Our results confirm that there are no substantial changes in the protein, the ring-shaped density, and most of the observed monoolein molecules (Fig. 1A). Importantly, this crystal was grown in LCP with monoolein, but with a different precipitating agent, pentaerythritol ethoxylate 15/04 (Fig. 1B). This bulkier, tetrahedral-shaped molecule could not substitute for the linear PEG 400 nor occupy any sites of electron density that Wang suggests as PEG 400 molecules. Specifically, it could not fit into the region that we have suggested might be a porphyrin

binding site. Yet, in the overlay of the two structures, the positions of various putative monooleins are essentially identical, although a few more are resolved in the 1.8-Å structure (Fig. 1A). In both structures, the extra ring-shaped density ($2F_{\text{obs}} - F_{\text{calc}}$) interpreted as a porphyrin is very similar (Fig. 1C) (2).

Identification of the ring-shaped density was also supported by biochemical data. As shown in the ultraviolet-visible spectrum in the supplementary materials of our Report (fig. S8A) (2), the absence of most of the Soret band [as also seen in (3)] and the presence of alpha bands in the 500- to 600-nm region is consistent with an oxidized form of porphyrin, as we suggested. Therefore, it was not expected that the modeled PpIX would fit the density very well. In our Report we stated, “A partially oxidized porphyrin is suggested by the spectrum (fig. S8A) that shows absence of a Soret band” (2). Wang also questions the non-planar nature of the fitted porphyrin, but there are many examples in the literature and structural databases with distorted and “ruffled” porphyrins [e.g., PDB 3QGT (4), PDB 4GPH (5), and PDB 3EEE (6)]. Distorted porphyrins are often found in proteins whose function is to degrade heme, where the distortion is thought to contribute to the mechanism. It has been proposed that one of TSPO's functions is to facilitate porphyrin breakdown (3), and as such, a distorted porphyrin in TSPO might be expected. Nevertheless, the high affinity binding of protoporphyrin IX to RsTSPO would still be observed by fluorescence quenching experiments because the oxidized porphyrin is present at less than 20% in the crystal structure (only one monomer out of three contains porphyrin, at <60% occupancy).

Wang's Comment states that at 32% in the crystallization medium, PEG 400 is the likely non-protein ligand within the crystal. However, Wang neglected to consider how LCP crystallization

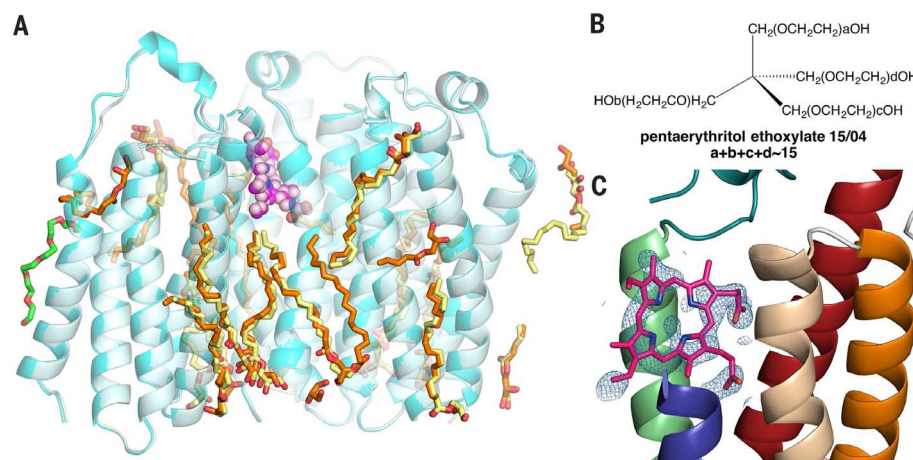


Fig. 1. Structure of RsTSPO-A139T obtained in pentaerythritol ethoxylate 15/04 is very similar to that obtained in PEG 400. (A) Structure of RsTSPO-A139T crystallized in PEG 400 (cyan) and pentaerythritol ethoxylate 15/04 (light cyan) show identical features of the porphyrin-like molecules (magenta and light magenta spheres) and most monooleins (orange and yellow sticks). A PEG molecule (green) could be fitted into the 1.8 Å structure but not in the 2.4 Å structure. One asymmetric unit containing three monomers is shown. (B) Structure of pentaerythritol ethoxylate 15/04. (C) Ring-shaped density ($2F_{\text{obs}} - F_{\text{calc}}$) and the fitting of a porphyrin-like molecule in the 2.4-Å structure obtained with pentaerythritol ethoxylate 15/04.

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Table 1. Statistics of x-ray data of RsTSP0-A139T. Diffraction data are processed with XDS package (11, 12). Statistics of the unmerged data are shown. I/σ , mean of intensity/sigma (intensity) of unique reflections (after merging symmetry-related observations); CC(1/2), percentage of correlation between intensities from random half data sets (correlation significance at the 0.1% level is marked by an asterisk); Anomal corr, percentage of correlation between random half-sets of anomalous intensity differences; Sig ano, mean anomalous difference in units of its estimated standard deviation ($|F(+)-F(-)|/\sigma$); Nano, number of unique reflections used to calculate Anomal corr and Sig ano.

Resolution limit	Number of reflections			Completeness of data (%)	R factor (%)		Compared	I/σ	R meas (%)	CC (1/2)	Anomal corr	Sig ano	Nano
	Observed	Unique	Possible		Observed	Expected							
2.21	29059	7599	7609	99.90	20.90	21.00	29059	7.13	24.30	96.6*	-1	0.782	4772
2.02	31920	8379	8391	99.90	39.10	39.30	31920	4.03	45.50	89.6*	0	0.751	5117
1.87	34512	9135	9145	99.90	81.80	80.90	34509	1.81	95.50	62.2*	0	0.717	5422
1.75	36929	9797	9807	99.90	151.50	156.30	36922	0.88	176.90	29.1*	0	0.646	5707
1.65	38230	10289	10420	98.70	272.90	293.00	38149	0.44	319.20	12.5*	0	0.589	5780
Total	244407	64547	64747	99.70	10.40	10.40	244313	9.03	12.10	99.8*	-1	0.731	39945

works. Within the lipidic cubic phase, the 60% (v/v) monoolein roughly corresponds to a concentration of 1.6 to 1.7 M, whereas the PEG 400 can only be as high as 0.35 M. Given that monooleins are critical for the stability of the LCP and the resulting membrane protein crystals, it is highly unlikely that the observed electron densities are correctly interpreted as PEG 400 molecules. Wang further contends that the ligand library density-fitting (LLDF) scores support his argument. However, the LLDF scores are known not to be suitable criteria for surface-bound ligands, such as lipids (7). A quick survey of membrane protein crystal structures determined in LCP with monoolein shows that most designated monooleins have an LLDF higher than 2, demonstrating that this criterion does not work well for lipidic molecules on the surface of the protein. (Structures surveyed include 4QND, 4N6H, 4JKV, 3VW7, 4E1Y, 4E1S, 3S8F, 3V5U, 2RH1, 3ZE3, and 4RYR.) On the other hand, we analyzed the re-refined structure of 4UC1 modeled with PEG provided by Wang using the PDB validation server. The result shows that most of the PEGs that Wang fitted into the transmembrane region in the place of monoolein have a LLDF score higher than 2 (as high as 206), which undermines his own argument.

However, we agree that raw data instead of merged data deposited in PDB provide more ac-

curate information on data quality. The chosen resolution cut-off of the data at 1.8 Å was supported by the statistics of the raw data. As shown in Table 1, data at 1.65 Å have very high R factors and very low I/σ and CC(1/2)—all indications of high noise—and therefore were excluded in the refinement. Wang has recommended (8) that data should be cut at I/σ around 1, which is ~1.8 Å in this data set, the same as the resolution we reported (2). The decision to limit our data set to 1.8 Å was also supported by the merging statistics for the raw data, which clearly revealed significant anisotropy in the highest shells (1.78 to 1.69 Å). This is quite evident in the analysis of the data by merging and scaling with the program Aimless (9); data within the resolution shell of 1.69 to 1.79 Å, the CC(1/2) for two of the principal directions drops precipitously to under 0.3, and the mean I/σ values have dropped well below 1. In addition, Xtriage from Phenix (10) shows that the data in the highest shells are very weak; less than 23% of the reflections have an $I/\sigma > 2$, and less than 2.6% have an $I/\sigma > 3$. Given that the data in the highest shells display a clear anisotropic distribution, there could be a significant anisotropic contribution to any added “detail” in the resulting electron density maps. This could potentially lead to misleading refinement results and interpretation. Thus, limiting our working data to 1.8 Å is a best-case compromise that adds

as much useful data as possible at high resolution but excludes the weak anisotropic data.

In conclusion, we believe that the new structure (5DUO) in pentaerithritol ethoxylate, along with chemical considerations and the statistics of the data, confirm our original conclusion that the best interpretation of the ring-shaped density is a porphyrin-type molecule and that the densities associated with the hydrophobic region of the protein are monooleins.

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The great Arctic experiment

Climate change is affecting the region's estuaries, politics, and what it means to go home

By **Gavin Stern**

Alaska's estuaries aren't home to many species. But a great diversity of life passes through these brackish, murky waters.

Climate change is altering these estuaries—which bridge freshwater ecosystems and the ocean—in ways that scientists lack the data to thoroughly understand. With the environment changing rapidly, there may not be much time to make baseline measurements.

“This is the experiment, the Arctic. Things are changing so fast, we may not be able to study it fast enough,” said Todd Radenbaugh, assistant professor in environmental science at the University of Alaska Fairbanks. “Estuaries are a conduit between terrestrial rivers, mountains, and the ocean. If you're a fish traveling through that, you must go through an estuary.”

The roles of species in these estuaries are not well understood, Radenbaugh said at the 2015 Arctic Science Conference, in large part because estuaries are difficult habitats to study. The environment is constantly changing with the tides and includes elements of both rivers and marine systems. Despite the unknowns, estuaries are among the most productive ecosystems in the world, used by many species for food, breeding, and migration.

The health and sustainability of estuaries in the face of climate change was the focus of the conference, the annual meeting of the AAAS Arctic Division. The University of Alaska Anchorage hosted the 1 to 3 October meeting, which was attended by researchers from the life, physical, and social sciences in addition to artists and educators.

The Arctic, which is warming twice as fast as in lower latitudes, according to the NOAA Arctic Report Card, is a bellwether for what will eventually occur in more populated southern regions, said Larry Duffy, executive director of the AAAS Arctic Division.

“What we see happening in the north within the biota and the physical environment will happen later at lower latitudes, but

with a much bigger impact,” said Duffy, who is also a professor of biochemistry at the University of Alaska Fairbanks. “When we talk here about a village of 500 people being eroded away, that's a problem. But when we talk about New York and New Jersey losing a portion of their coast due to sea-level rise—that's a big problem.”

In Alaska's estuaries, climate change affects more than just water temperature. Melting glaciers change the water's chemistry and influence what species can survive, both in the ocean and further inland. Fish are particularly sensitive to chemical changes, which can affect how they navigate, Radenbaugh said.

Many of the scientists at the meeting agreed that the ecological effects of climate change on estuaries need further study and that these environments must be monitored. Since scientists don't have a good way to make predictions, they said that it is difficult to develop policies flexible enough to adapt to changing conditions, such as when to catch fish and when to let stocks recuperate.

The unknowns will have both tragedies and positive opportunities associated with them, said plenary speaker Daniel Schindler, an ecologist and professor at the University of Washington.

“We can make decent predictions about physical systems like the atmosphere and oceans. But our track record in ecological systems is pretty meager,” Schindler said. “What we want is the ability to maintain flexibility so when one fish stock unexpectedly explodes, we have policies that let people go catch them, make a living off them, and eat them. Everything cannot be bad—there's no way.”

HOW BEST TO ADDRESS GLOBAL CLIMATE CHANGE is to a large extent a political issue, with the East and West arguing over who is most responsible for greenhouse gas emissions. That argument is expected to take center stage at the 2015 United Nations Climate Change Conference in Paris beginning on 30 November.

Cumulatively, the United States and the European Union countries are responsible for about half of the emissions produced since the Industrial Revolution, while China lays claim to 11%, said plenary speaker Alexios Antypas, director of the Center for Environment and Security at Central European University in Hungary, citing data from the World Resources Institute.

But today, China is “by far” the biggest emitter of carbon dioxide, Antypas said, responsible for 24% of the global greenhouse gas output in 2011. That’s about the same emissions as the United States (15%) and European Union (10%) combined.

“This is a real different place where we’re at now. It tells you a lot about the bargaining power. By far the most powerful actor of this is China,” Antypas said, noting that China and India will be responsible for much of the future load.

“Everyone else is either staying the same, growing slowly, or gradually decreasing,” Antypas said, including the United States, which has seen a decline back to 1994 emissions levels.

But when adjusting for population, the most responsible party becomes less clear. With a population of 34.3 million, Canada was the clear frontrunner in per capita emissions in 2011, followed by the United States, Russia, Japan, and the European Union.

The Chinese are well aware that much of their greenhouse gas output is due to American companies outsourcing their production, Antypas said. Fortunately, there are two clear leaders who can shape emissions policies.

“The rest of the world will go where the U.S. and China go—if they go together,” Antypas said. “China is in a position to lead the rapidly developing countries, and the U.S. is in a position to lead the developed countries.”

SEVERAL SESSIONS AT THE CONFERENCE were dedicated to the effects of climate change on human settlements. About 4 million people call the Arctic their home, many in indigenous communities that have lived in connection with the land for many years. They rely in large part on subsistence hunting and fishing.

As melting ice leads to new possibilities for shipping, drilling, and other industrial activities, “a lot of people are looking at the

economic opportunities. But people up here are looking at some very significant threats,” said Diane Hirshberg, director of the Center for Alaska Education Policy Research at the University of Alaska Anchorage. “The immediate change has already happened for indigenous communities—people falling through the ice when they’re using traditional ice routes. Whalers have lost their lives or had to be rescued because the ocean is unpredictable. Animals aren’t migrating in the same patterns, which causes food insecurity.”

Arctic communities are facing an increased frequency and severity of extreme weather events, changes in seasonality, and impacts on their terrestrial and marine ecosystems. Traditional ice paths are disappearing, tree lines are increasing, and riverbanks are eroding, according to Mary Dallas Allen, associate professor at the University of Alaska Anchorage School of Social Work.

Later freezing and earlier breakup of ice disrupts travel, infrastructure, and subsistence hunting activities. Food that is traditionally stored in cellars is going bad. Parents are deciding that it’s not safe to take their children on hunting and other traditional food-gathering outings, resulting in cultural loss.

Arctic communities are losing what it means to be home, Allen said, which is having significant social, emotional, and psychological effects.

Even for children to get to school, many communities rely on stable ice to get across rivers that have always been frozen, Allen said. With unstable ice, children miss school. Or, getting to school is scary and unsafe.

“Climate change is affecting people’s lives every day,” Allen said. “When people are losing homes, livelihoods, and the safety net of their communities, there are associated mental health challenges. They experience depression, anxiety, posttraumatic stress, increased substance abuse, and higher rates of suicide.” ■



Climate change takes a psychological toll on Arctic communities, said social worker Mary Dallas Allen.

Awards honor early-career women in the chemical sciences

The inaugural AAAS Marion Milligan Mason awards recognized four outstanding chemists and mentors

By **Kathy Wren** and **Andrea Korte**

Luisa Whittaker-Brooks wants to create a material that can be used to harvest ambient heat—from our bodies, for example—and turn it into electricity.

This material will need to be a good electrical conductor, but it mustn’t be thermally conductive or the heat would escape from the system. So Whittaker-Brooks, an assistant professor of chemistry at the University of Utah, is working on integrating organic and inorganic materials—essentially, plastics and semiconductors—at the nanoscale. Because these two types of materials “don’t like each other,” as she says, Whittaker-Brooks must manipulate the interfaces between the particles to encourage them to mingle.

This is just part of a master plan, whose ultimate goal is creating a device that can capture and store both thermal and solar energy, to continuously produce electricity. Such ambitious research can be tricky for early-career scientists, who too often find themselves in a Catch-22-like situation with regard to funding, especially when they want to do “high-risk, high-reward” research that could potentially lead to major breakthroughs.

“As an early-career scientist, this is very difficult at times, to get the funding for high-risk research,” said Alison Fout, an assistant professor of chemistry at the University of Illinois at Urbana-Champaign. “We need preliminary results, we need data, we need publications, and then we need to get that funding. And as you can imagine, all that takes time, and time costs money.”

Fout and Whittaker-Brooks now have the means to tackle this problem, however, because they and two other early-career chemists have won the first AAAS Marion Milligan Mason Awards for Women in the Chemical Sciences. The group—which also includes Kristin Parent, an assistant professor of biochemistry and molecular biology at Michigan State University, and Katherine Mackey, an assistant professor of Earth system science at the University of California, Irvine—was recognized at a 15 October award ceremony at AAAS.

The awards, made possible by a \$2.2 million bequest to AAAS, provide each chemist with \$50,000 to ramp up their research projects while mentoring their own students.

A chemist and long-time AAAS member, the late Marion Tuttle Milligan Mason wanted to support the advancement of women in



From left: Rush Holt, Shirley Malcom, Geri Richmond, Luisa Whittaker-Brooks, Kristin Parent, Katherine Mackey, Alison Fout, and AAAS Board member Laura Greene.

the chemical sciences. She also wanted to honor her family's commitment to higher education for women, as shown by her parents and her grandfather, who sent several daughters to college.

After graduating from Vassar College in 1949, Mason worked as a chemist at the American Cyanamid Company. When she learned that, compared to her colleagues who had recently earned doctoral degrees in chemistry, she was performing similar work for considerably less compensation, she felt motivated to continue her education, and in 1970, Mason earned her Ph.D. in organic chemistry from Rutgers University.

"I am creating this fund in honor of the memory of all the men and women of the Tuttle and Milligan families who believed in higher education for women and encouraged them in their pursuit of professional and business careers," Mason wrote in her will.

The fund will provide grants every 2 years, for the next 20 years, to outstanding women researchers in the chemical sciences. "Marion Milligan Mason understood how she had benefited from being born into a family that understood the importance of and supported the science education of women. It is that legacy that we come here to recognize and to continue," said Shirley Malcom, director of AAAS Education and Human Resources.

While the numbers of women entering STEM careers, including faculty positions in academia, has been growing, women are still, along with minorities and persons with disabilities, under-represented in these fields. The disparities are particularly stark in chemistry and other physical sciences, where just 30% of the jobs in these fields are held by women, according to the National Science Foundation.

"These are serious issues, and until we change the climate at our institutions and develop policies that will help these women—not just women, but anyone who feels disadvantaged—our innovation will suffer and our nation will suffer," said AAAS President Geri Richmond, who is also a U.S. science envoy and presidential chair and professor of chemistry at the University of Oregon.

At the ceremony, each of the winners thanked their mentors and supporters, and gave a brief description of their research.

While Whittaker-Brooks is a materials chemist, Fout and the other winners are working on topics that blend chemistry and biology.

Fout builds synthetic and organic molecules and studies models of them in her lab. One of the questions she is investigating is how hemoglobin reduces nitrite, a process that affects blood pressure.

Parent uses cryoelectronic microscopy to determine the three-dimensional structure of large, complex viruses. This information sheds light on how viruses recognize and infect their hosts.

Mackey studies how marine phytoplankton respond to changing nutrient and light conditions. She is particularly interested in how climate change is affecting these microscopic organisms, as the oceans become more acidic and the vertical mixing of water layers becomes more sluggish.

"The life of an early-career scientist has a lot to do with writing grants and hoping you get funding so that you can get those students

and support them," said Mackey. "This grant will enable me to go out and get my students involved in fieldwork right away so that I can have the same effect on them that my mentors have had on my career." ■

AAAS Council reminder

The next meeting of the AAAS Council will take place during the 2016 AAAS Annual Meeting in Washington, DC and will begin at 9:00 a.m. on 14 February 2016 at the Omni Shoreham Hotel.

Individuals or organizations wishing to present proposals or resolutions for possible consideration by the council should submit them in written form to AAAS Chief Executive Officer Rush Holt by 1 December 2015. This will allow time for them to be considered by the Committee on Council Affairs at its winter meeting.

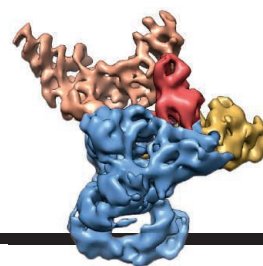
Items should be consistent with AAAS's objectives and be appropriate for consideration by the council. Resolutions should be in the traditional format, beginning with "Whereas" statements and ending with "Therefore be it resolved."

Late proposals or resolutions delivered to the AAAS Chief Executive Officer in advance of the February 2016 open hearing of the Committee on Council Affairs will be considered, provided that they deal with urgent matters and are accompanied by a written explanation of why they were not submitted by the 1 December deadline. The Committee on Council Affairs will hold its open hearing at 2:30 p.m. on 13 February 2016 in the Congressional Room of the Omni Shoreham.

RESEARCH

Telomerase complex reveals its secrets

Jiang et al., p. 529



IN SCIENCE JOURNALS

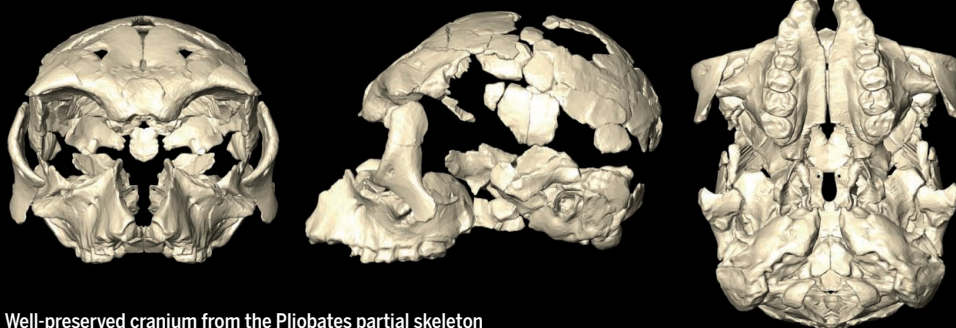
Edited by Stella Hurtley

PRIMATE EVOLUTION

Meet your gibbon cousin

Apes are divided into two groups: larger-bodied apes, or hominoids, such as humans, chimps, and gorillas; and smaller-bodied hylobatids, such as gibbons. These two lineages are thought to have diverged rather cleanly, sharing few similarities after the emergence of crown hominoids. Alba *et al.* describe a new ape from the Miocene era that contains characteristics from both hominoids and small-bodied apes (see the Perspective by Benefit and McCrossin). Thus, early small-bodied apes may have contributed more to the evolution of the hominoid lineage than previously assumed. — SNV

Science, this issue p. 528; see also p. 515



Well-preserved cranium from the *Pliobates* partial skeleton

BATTERIES

Solving the problems with Li-air batteries

Li-air batteries come as close as possible to the theoretical limits for energy density in a battery. By weight, this is roughly 10 times higher than conventional lithium-ion batteries and would be sufficient to power cars with a range comparable to those with gasoline engines. But engineering a Li-air battery has been a challenge. Liu *et al.* managed to overcome the remaining challenges: They were able to avoid electrode passivation, turn limited solvent stability into an advantage, eliminate the fatal problems caused by superoxides, achieve high power with negligible

degradation, and even circumvent the problems of removing atmospheric water. — MSL

Science, this issue p. 530

GEOMORPHOLOGY

Bedrock weathering runs to the hills

Fractures in bedrock drive the breakdown of rock into soil. Soil makes observations of bedrock processes challenging. St. Clair *et al.* combined a three-dimensional stress model with geophysical measurements to show that bedrock erosion rates mirror changes in topography (see the Perspective by Anderson). Seismic reflection and electromagnetic profiles allowed mapping of the bedrock

fracture density. The profiles mirror changes in surface elevation and thus provide a way to study the critical zone between rock and soil. — BG

Science, this issue p. 534; see also p. 506

MAGNETISM

Visualizing conducting domain walls

When a metal undergoes a phase transition and becomes insulating, it sometimes also becomes magnetically ordered. It is possible that some metallicity survives along the boundaries of magnetic domains, the so-called domain walls, but the question is difficult to address directly in experiments. Ma *et al.* did just that by mapping

out the conductance of the material $\text{Nd}_2\text{Ir}_2\text{O}_7$ in its low-temperature magnetic insulating phase, using microwave impedance microscopy. The magnetic domain walls showed up clearly in the images as regions of high conductance. — JS

Science, this issue p. 538

CHRONIC ITCH

A circuit controlling mechanical itch

Considerable progress has been made in understanding and treating chemically induced itch. However, little is known about the mechanisms underlying mechanically evoked itch. Bourane *et al.* produced a model of mechanical itch by reducing the number of neuro-peptide Y-expressing inhibitory spinal interneurons. This led to a selective increase in mechanically evoked itch-like behavior in mice. In contrast, chemically evoked itch or pain behavior remained unaffected. — PRS

Science, this issue p. 550

VIROLOGY

A close up view of retrovirus spreading

Viral infections typically begin with a small number of viral particles gaining access to the host at a specific tissue site. But how do viruses that cause systemic infections, such as HIV, spread more widely? Sewald *et al.* visualized how the retroviruses murine leukemia virus (MLV) and HIV spread within lymph nodes in mice (see the Perspective by Hope). Specific macrophages that line

the lymph-draining sinuses in lymph nodes first captured the virus using the carbohydrate-binding protein CD169. These macrophages subsequently transferred virus to the B1 subclass of B lymphocytes, which migrated further into the lymph node, disseminating the virus more widely. — KLM

Science, this issue p. 563;
see also p. 511

LUNG DISEASE

Anticoagulant “morfs” into pneumonia therapy

Pneumonia can cause lung cell death, yet the mechanisms by which infection reduces cell viability are unclear. Zou *et al.* found that a poorly described protein, Morf4l1, triggers cell death in mice with pneumonia. The half-life of Morf4l1—normally a short-lived protein—was increased in the context of pneumonia. The anticoagulant drug Argatroban blocked half-life extension as well as the injurious actions of Morf4l1, thus prolonging the survival of mice with experimental pneumonia. — OMS

Sci. Transl. Med. **7**, 311ra171 (2015).

ECONOMICS

Comparing lab and field estimates

What do Swiss high-school students and Eastern European seasonal laborers have in common? When the former are tasked with stuffing

questionnaires into envelopes in a classroom setting and the latter are employed to pick fruit in the United Kingdom, both work harder in the presence of their peers. Herbst and Mas reanalyzed the results of 35 such studies, either experiments carried out under controlled conditions or empirical studies based on data collected in the field (see the Perspective by Charness and Fehr). Encouragingly, they found that the magnitude of the spillover effect—how much harder a worker works when other workers are alongside—was the same. — GJC

Science, this issue p. 545;
see also p. 512

REPRODUCTIVE BIOLOGY

Ensuring a speedy labor, STAT!

The amnion is one of the membranes that surround the fetus. In the amnion, cortisol enhances the activity of the enzyme COX-2, which produces prostaglandins that trigger changes in uterine smooth muscle that enable delivery. Wang *et al.* used amnion fibroblasts from full-term births from women who delivered after active labor or through cesarean section (see the Focus by Zannas and Chrousos). They found that the glucocorticoid receptor interacted with the transcription factor STAT3 to promote transcription of the gene encoding COX-2, amplifying cortisol-triggered prostaglandin production. — LKF

Sci. Signal. **8**, ra106; fs19 (2015).

IN OTHER JOURNALS

Edited by **Kristen Mueller**
and **Jesse Smith**



By swapping portions of their genomes, *Penicillium* species optimized themselves for cheesemaking

MICROBIOLOGY

Genomes share shortcuts to cheese

Making cheese requires fermentation, which means that it can be a messy business. To better understand the process at a molecular level, Ropars *et al.* analyzed the genomes of several *Penicillium* fungal species, including those used to make Roquefort and Camembert cheese. Different species shared large portions of their genomes, as much as 80 kilobases in size. The genes embedded in these shared islands helped the fungi to break down milk components more effectively and compete with other microbes. The fungi probably acquired these genomic islands through horizontal gene transfer rather than by reproducing. During the centuries that humans have been selecting fungi to make better cheese, the fungi have apparently been passing notes under the table to get the job done. — PJH

Curr. Biol. **25**, 2562 (2015).

C-H BOND ACTIVATION

Threading the needle with manganese

It's rather straightforward to oxidize hydrocarbons indiscriminately—that's the type of reaction that supplies heat

to gas-powered stoves and furnaces. Chemists interested in making pharmaceuticals or consumer products do not want to end up with just carbon dioxide and water though. Instead, they want to transform certain specific C-H bonds



Fruit pickers collect the harvest in the United Kingdom

PHOTOS: (FROM TOP) © ADAM WOOLFE/CORBIS; JOHN GILES/PA WIRE

ALSO IN SCIENCE JOURNALS

Edited by Stella Hurtley

RISK ASSESSMENT

Setting policy, knowing risks

Policy-makers often commission formal analyses to estimate the costs, risks, and benefits of proposed projects or policies. Applications range from estimating the risks of commercial nuclear power, to setting priorities among environmental risks, to comparing technologies for generating electricity, to weighing the benefits and risks of prescription drugs. In the United States, analyses are required for all major federal regulations. Fischhoff reviews how such analyses are limited by the scientific and ethical judgments inherent in the process and require collaboration between those who generate the analyses and those who want to use them. — BJ

Science, this issue p. 527

STRUCTURAL BIOLOGY

Chromosome-capping enzyme complex

Telomeres cap and protect the ends of our chromosomes. The telomerase complex helps maintain the telomere DNA repeat sequences. Telomerase consists of an RNA and a number of protein subunits. Jiang *et al.* used cryo-electron microscopy and x-ray crystallography to determine the structure of the *Tetrahymena* telomerase complex. The telomerase is made up of three subcomplexes, which include two previously unknown protein subunits in addition to the seven known subunits. The structures also reveal the path

of the RNA component in the telomerase catalytic core. — GR
Science, this issue p. 529

SUPERCONDUCTIVITY

Cooling to see the effects of disorder

In sufficiently strong external magnetic fields, thin superconducting films typically become insulating. The presence of disorder can affect this phase transition. Theorists have proposed that disorder can cause the so-called Griffiths singularity, where the behavior of the system is determined by a small number of superconducting islands that form above the critical magnetic field. Xing *et al.* observed a signature of such a singularity in thin films of gallium by analyzing transport data taken at very low temperatures (see the Perspective by Markovic). In this regime, thermal fluctuations were not strong enough to homogenize the system, which allowed the rare islands to form. — JS

Science, this issue p. 542;
see also p. 509

BRAIN DEVELOPMENT

Circuits for brain building

The brain is made up of circuits. Chen and Kriegstein analyzed how one particular circuit functions differently in developing and mature mouse brains (see the Perspective by Spitzer). This circuit connects the brain's diencephalon to the cortex. Early in development, the circuit is excitatory and affects how the

cortical neurons it touches build their dendrites and synapses. Later on, after the fundamentals of brain construction are in place, the circuit switches to inhibitory functions, helping the brain resist epileptiform activity. — PJH

Science, this issue p. 554;
see also p. 510

MICROBIOME

The benefits of *Escherichia coli*

Infection and intestinal damage can trigger severe muscle wasting and loss of fat in mice. How this happens is poorly understood. Palaferri Schieber *et al.* discovered a protective *Escherichia coli* strain in their mouse colony. Mice intestinally colonized with the *E. coli* and infected with the food-poisoning bug *Salmonella* or with the lung pathogen *Burkholderia* did not waste away. Without the *E. coli*, similarly infected mice became fatally ill. The protective *E. coli* stimulated an innate immune mechanism that ensured that muscle-signaling pathways were not damaged by infection. Thus, the friendly *E. coli* allowed its host to tolerate and survive the pathogens. — CA

Science, this issue p. 558

TUMOR VIRUSES

Viral oncogenes remove the host's STING

Cancer-causing viruses, such as the human papilloma virus (HPV) that causes cervical cancer, account for 12% of human cancers. One way they can cause cancer is by targeting

tumor suppressor proteins in the host. Now Lau *et al.* report that DNA tumor viruses can also thwart the host's immune system. Oncogenes from HPV and human adenovirus bound to the protein STING, a key component of the cGAS-STING pathway that senses and defends against intracellular DNA. In this way, the viruses subvert the host's antiviral immunity and set up shop, which, for an unlucky few, eventually causes cancer. — KLM

Science, this issue p. 568

ECOLOGY

Reversing the butterfly effect

In the 1970s, "Does the flap of a butterfly's wings in Brazil set off a tornado in Texas?" was a provocative seminar title that reached the public psyche. What of the converse? How will climate change affect butterfly populations? Palmer *et al.* extended the strategy of looking at bellwether species to bellwether taxa, in order to better analyze the impact of 40 years of climate variability on the abundance of 23 butterfly and 131 moth species. Over half of the changes in the abundance of a species across the United Kingdom reflected species-specific exposure and sensitivity to climate. The findings suggest that we need to rethink early-21st-century assumptions about the effect of climate change on butterflies; many species thought to benefit from warming trends will in fact decline in abundance. — BJP

Sci. Adv. 10.1126/sciadv.1400220 (2015).

without disturbing the rest of the molecule. Paradine *et al.* report a manganese catalyst that slides pendant N into C-H bonds with high tolerance for otherwise-reactive C=C double bonds nearby. At the same time, the catalyst shows surprisingly high reactivity toward very strong C-H bonds (including primary alkyl sites) that previous selective catalysts failed to engage. — JSY

Nat. Chem. 10.1038/NCHEM.2366 (2015).

AGING

Skin cell-derived neurons act their age

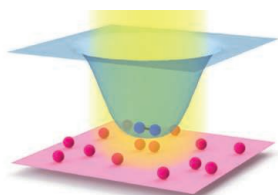
The main risk factor for neurodegenerative disorders is aging. To better understand cellular aging, scientists seek to model it using human neurons in tissue culture. Given the difficulty of obtaining neurons directly from human donors, scientists can derive them from either induced pluripotent stem cells (iPSCs) or by directly inducing them from another cell type. Mertens *et al.* compared the gene signatures of neurons obtained by these two methods and found that although the iPSC-derived neurons erased their signatures of aging, the induced neurons retained their original aged characteristics. Thus, directly converted induced neurons could provide a key resource for modeling neuronal aging. — SMH

Cell Stem Cell 10.1016/j.stem.2015.09.001 (2015).

PHYSICS

Tweaking atomic interactions optically

Control over interactions in ultracold atomic gases makes them an appealing setting for



Schematic view of the optical control of Feshbach resonances

quantum simulations. To exert this control, researchers typically tune external magnetic fields. However, varying interactions in time and space using this method remains tricky. Now, Clark *et al.* implement an optical scheme that enables such modulations. By carefully choosing the wavelength of the laser light they shone at a gas of Cs atoms, the researchers were able to modulate the interactions without the side effects that plagued earlier approaches. The work extends the potential of cold gases for quantum simulation. — JS

Phys. Rev. Lett. 115, 155301 (2015).

ATMOSPHERIC CHEMISTRY

A suite in flux

Understanding the chemistry of the atmospheric boundary layer is an exercise that requires knowledge of a tremendous number of chemical species, their interactions with one another, and how those interactions vary under different physical conditions. To date, most observational efforts have focused on measuring the concentrations of the most important gases, but such limited information makes it difficult to construct a model of the atmosphere that is at once quantitative, comprehensive, and self-consistent. Wolfe *et al.* improve on existing methodologies by developing an airborne system capable of quantifying not only concentrations but fluxes for a suite of reactive gases. Their approach will improve our knowledge of biogenic and anthropogenic emissions, photochemical mechanisms, and deposition. — HJS

Geophys. Res. Lett. 10.1002/2015GL065839 (2015).

CELL AGING

Yeast reveal the secrets for a long life

For scientists, the genetic basis of aging largely remains a mystery. McCormick *et al.* sought

PSYCHOLOGY

Trusting robots but not androids

R

obots collect warehoused books, weld car parts together, and vacuum floors. As the number of android robots increases, however, concerns about the “uncanny valley” phenomenon—that people dislike a vaguely human-like robot more than either a machine-like robot or a real human—remain. Mathur and Reichling revisited whether human reactions to android robots exhibit an uncanny valley effect, using a set of 80 robot head shots gathered from the Internet and a systematically morphed set of six images extending from entirely robot to entirely human. Humans did adhere to the uncanny valley curve when rating the likeability of both sets of faces; what’s more, this curve also described the extent to which those faces were trusted. — GJC

Cognition 146, 22 (2016)

Robots that resemble humans often make people uncomfortable.

to shed light on this mystery by conducting a 10-year study of the replicative life span (that is, the number of daughter cells that a given cell produces) in 4698 strains of yeast, each of which had a different gene deleted. The authors detected 238 genes that promoted longevity when deleted. They then grouped them into functional

categories, the largest being genes that encode components of the ribosome, a large molecular machine that drives protein synthesis. Many genes associated with yeast longevity overlapped with those that regulate this process in *Caenorhabditis elegans*. — BJ

Cell Metab. 10.1016/j.cmet.2015.09.008 (2015).

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PHOTOS: (FROM LEFT) CLARK ET AL., PHYSICAL REVIEW LETTERS 115 (9 OCTOBER 2015) © PHYSICS; MAX AGUILERA-HELLWEG

REVIEW SUMMARY

RISK ASSESSMENT

The realities of risk-cost-benefit analysis

Baruch Fischhoff

BACKGROUND: Synthetic biology, nanotechnology, geoengineering, and other innovative technologies share a property: Their effects must often be inferred long before they are experienced. If those inferences are sound, then informed decisions are possible. If not, then decision-makers may incur risks and costs far greater than any expected benefits. Risk, cost, and benefit analysis can offer

transparent ways to assemble and integrate relevant evidence to support complex decision-making. All forms of analysis have the same logic: Decompose complex systems into manageable components and then calculate how they might perform together. All require scientific judgment to bound the set of components and assess the limits to those bounds. All require ethical judgment to determine

which outcomes to predict and to extract the policy implications of the results. The usefulness of any analysis depends on how well its underlying assumptions and their implications are understood by those hoping to

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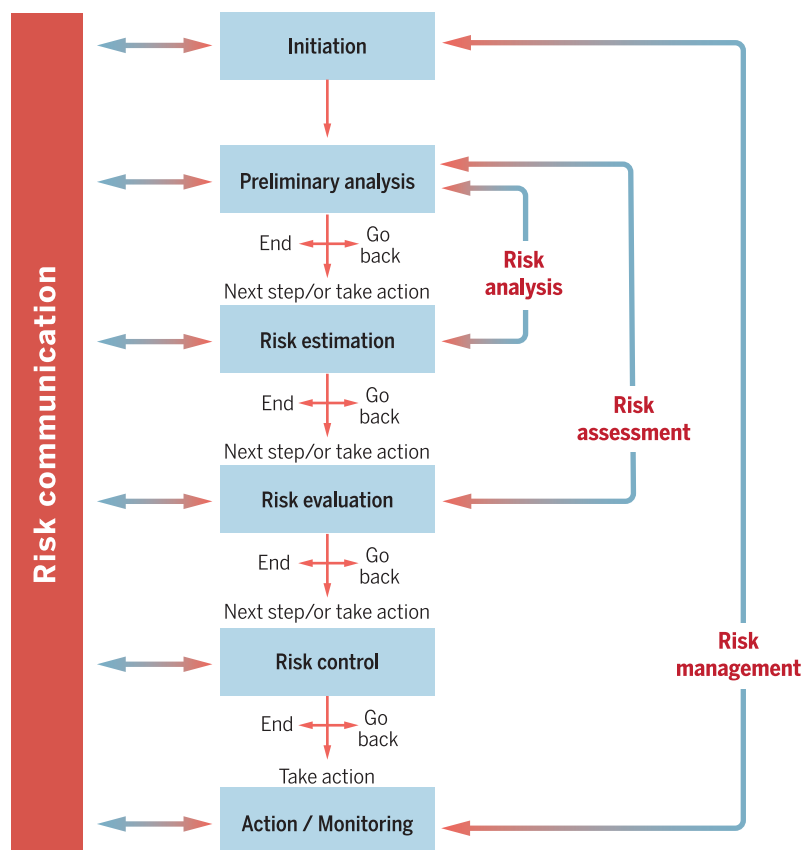
Read the full article at <http://dx.doi.org/10.1126/science.aaa6516>

use its results. The present review uses historical examples to illustrate the roles of judgment in analyses that address four basic questions: (i) How large are the risks from

a single technology? (ii) Which risks merit the greatest attention? (iii) Which technology produces the least risk per unit of benefit? (iv) Are a technology's expected benefits acceptable, given its risks and other expected costs?

ADVANCES: Analyses are always incomplete. They neglect concerns that are hard to quantify. They define terms in ways that serve some interests more than others. They consider some sources of uncertainty but not others. Advances in the science of analysis have often occurred after critics unhappy with the results of an analysis challenged the legitimacy of its assumptions. Awareness of the role of judgment in analysis has grown over time, in parallel with improvements in the sophistication of analytical calculations. Progress has been made in some areas, but more is needed, to include developing better ways to model human behavior, elicit expert judgments, articulate decision-makers' preferences, characterize the robustness of conclusions, and communicate with decision-makers. The practice of analysis draws on the sciences of public participation and science communication, both shaped by the challenges faced in securing a fair hearing for science in issues where it plays a central role.

OUTLOOK: The pace of advances will depend on the degree of collaboration among the sciences relevant to these problems, including not only the sciences underlying the technology in question but social, behavioral, and economic science as well. How well the science of analysis aids its practice will depend on how well analysts collaborate with decision-makers so as to produce the estimates that decision-makers need and ensure that analytical results are properly understood. Over time, those interactions will help decision-makers understand the capabilities and limitations of analysis while helping analysts become trusted allies, dedicated to producing relevant, properly qualified estimates of cost, risk, and benefit. ■



An analytical-deliberative process in which analysts and decision-makers collaborate in managing risks. The process begins by defining the terms of the analysis (initiation), proceeds to preliminary analysis, identifying the issues meriting greatest attention, and continues through estimation of the magnitude of the risks, evaluation of their acceptability, and consideration of control mechanisms, improving the risk-cost-benefit trade-offs. Once an action has been selected, monitoring assesses how well the ensuing reality corresponds to the analytical conclusions. At all stages, analysts communicate with those potentially affected by the risks in question. Analogous processes apply to cost and benefit analyses. [Adapted from Canadian Standards Association, *Risk Management: Guidelines for Decision Makers* (Q850) (CSA, Ottawa, Canada, 1997)]

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REVIEW

RISK ASSESSMENT

The realities of risk-cost-benefit analysis

Baruch Fischhoff

Formal analyses can be valuable aids to decision-making if their limits are understood. Those limits arise from the two forms of subjectivity found in all analyses: ethical judgments, made when setting the terms of an analysis, and scientific judgments, made when conducting it. As formal analysis has assumed a larger role in policy decisions, awareness of those judgments has grown, as have methods for making them. The present review traces these developments, using examples that illustrate the issues that arise when designing, executing, and interpreting analyses. It concludes with lessons learned from the science and practice of analysis. One common thread in these lessons is the importance of collaborative processes, whereby analysts and decision-makers educate one another about their respective needs and capabilities.

Formal analyses are often commissioned to estimate the costs, risks, and benefits of projects or policies. As seen below, the range of applications is as diverse as estimating the risks of commercial nuclear power, setting priorities among environmental risks, comparing technologies for generating electricity, and weighing the benefits and risks of prescription drugs. In the United States, analyses are required for all major federal regulations. One current analysis is examining the risks of gain-of-function research for pathogens with pandemic potential (i.e., studying how they could become more potent), hoping to resolve a dispute among biological scientists (1).

Risk, cost, and benefit analysis reflect a strategy of bounded rationality (2). Rather than attempting to address all aspects of a complex decision, such analyses “bound” it, in the sense of ignoring enough of its elements to be able treat those that remain “rationally.” Typically, that means estimating the expected effect of each decision option by multiplying the size of possible outcomes by their probability of occurring should the option be chosen.

Whether those calculations lead to better decisions depends on how well two sets of judgments are made and understood. One set comprises the ethical judgments involved in defining “risk,” “cost,” and “benefit,” thereby specifying which outcomes are deemed worth estimating. The second set comprises the scientific judgments involved in recruiting and interpreting the evidence used in estimating those outcomes (3–7).

All analyses have potentially controversial formulations, reflecting the ethical judgments underlying them, and uncertain conclusions, reflecting the scientific ones. For example, an ethical judgment

determines whether an analysis estimates just the total risks, costs, and benefits for all people affected by a decision or if it also considers distributional effects, reflecting how those outcomes differ across groups of people (e.g., rich people versus poor people or people today versus people tomorrow). A scientific judgment determines whether an analysis considers just physical processes affecting the outcomes (e.g., valve failures and toxic plumes) or also human factors (e.g., how well workers operate equipment or how faithfully patients take medications). To use analyses wisely, decision-makers need to know what judgments were made and how they affected the results.

Awareness of such ethical and scientific judgments has grown slowly over the history of analysis, often emerging when motivated critics claimed to have found flaws in analyses whose results displeased them. The present review uses historical examples to illustrate the roles of judgment in analyses that address four basic questions: (i) How large are the risks from a single technology? (ii) Which risks merit the greatest attention? (iii) Which technology produces the least risk per unit of benefit? and (iv) Are a technology’s expected benefits acceptable, given its risks and other expected costs?

The science of analysis

Analysis is not a science, in the sense of formulating and evaluating general theories. However, analyses rely on scientific results to guide the judgments needed when setting bounds, calculating estimates, and assessing their robustness. For example, when evaluating a new drug, analysts’ ethical judgments might be informed by research into which benefits and side effects have the greatest effect on patients’ lives; their scientific judgments might be informed by research into how likely patients are to take the drug as prescribed.

Exercising scientific judgment when gathering and interpreting evidence is a task faced by both analysts and scientists. Where analysts’ work differs from that of scientists is in the breadth of their evidence-gathering and the length of their interpretative chains. For example, analysts estimating the environmental impacts of a genetically modified crop must gather evidence regarding ecology, entomology, agronomy, and industrial chemistry, among other things, and then must project the implications of that evidence into a future world with potential changes in climate, land use, trade, and regulation, among other things. Whereas scientists might consider one or two interactions among such factors (e.g., how a longer, drier growing season might affect a pest), analysts might need to consider them all.

The science of analysis develops general methods for performing these tasks. Those methods include procedures for eliciting expert judgments (when observations are lacking), for combining diverse forms of evidence, and for assessing residual uncertainties (3, 7–10). The science of analysis has also developed methods for making ethical judgments (3–5). Those methods include procedures for eliciting individuals’ preferences directly and for inferring those preferences from their behavior (11, 12). As seen in the examples that follow, the science of analysis, like other sciences, has often advanced in response to challenges to controversial results [e.g., (6, 13–15)].

Analyzing risks from one source: Nuclear power

In 1972, facing public concern over the risks of commercial nuclear power, the Atomic Energy Commission sponsored a probabilistic risk analysis of pressurized water reactors. In 1975, Norman Rasmussen and colleagues delivered WASH-1400, the Reactor Safety Study (16). Building on work in the chemical, aerospace, and other industries (17, 18), WASH-1400 sought to identify all major accident sequences and then calculate the probability of each sequence occurring and the expected number of immediate deaths should that happen. It used both forward-looking event-tree analysis, asking how accidents could arise from normal operations, and backward-looking fault-tree analysis, looking for precursors of possible accidents.

WASH-1400 greatly advanced the science of risk analysis. However, rather than resolving the question of nuclear safety, it sparked intense controversy. Reviews from the American Physical Society and the Nuclear Regulatory Commission, examining the scientific judgments underlying WASH-1400 (19, 20), concluded that there was no reason to believe that the report’s risk estimates were biased upward or downward. However, the reviewers were confident that those estimates had been stated with unwarranted certainty. Moreover, they could not say by how much (20). One source of the reviewers’ uncertainty was “that WASH-1400 is inscrutable...it is very difficult to follow the detailed thread of any calculation through the report” [(20), p. vii].

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Even though they could not repeat the study's calculations, the reviewers could still audit it for structural problems, in the sense of seemingly relevant factors left outside its bounds. Those omissions included risks related to evacuation and manufacturing, as well as "common cause failures," whereby an initiating event (e.g., a tsunami, earthquake, or terrorist attack) damages systems meant to provide redundant protection. The poor documentation was in itself troubling. If the reviewers could not follow the work, how well could the analysts have stayed on top of it?

The reviewers commended the study for considering the effects of operator behavior on reactor safety rather than examining only physical factors. Nonetheless, they believed that the analysts' scientific judgment had overestimated those risks by underestimating "human adaptability during the course of an accident" [(20), p. vi].

The formal analysis of human behavior received a boost, a few years later, as a result of operators' apparent role in the Three Mile Island accident of 1979. One approach has been human reliability analysis, which applies probabilistic risk analysis to human behavior (21). Such computational methods are potentially useful for estimating failure rates with highly structured tasks, like the assembly-line munitions production for which they were initially developed. Unfortunately, it is another matter to produce quantitative estimates of the risks arising from human factors in cognitively intense tasks, such as the design, operation, and management of complex systems (22–25). On the other hand, when the need to calculate everything is relaxed, the logic of analysis can clarify the tasks facing operators, anticipating and perhaps avoiding problems (22, 26, 27).

Although WASH-1400's omissions compromised its overall risk estimates, its wide scope still produced "increased understanding of the full spectrum of reactor accident sequences [with] implications for nuclear power plant design, siting, and planning for mitigation of consequences" [(20), p. ix]. Thus, the study may have identified ways to make nuclear power safer, even though it could not establish how safe the technology was overall. Assessing the relative risk of alternative designs is a more tractable task analytically than estimating any one design's absolute risk level (28). Comparing designs requires examining only those elements where they differ. Estimating any single design's overall risk requires examining every element in every accident sequence, however hard to quantify (e.g., terrorists' plans for attacking reactors).

Although it focused on the study's scientific judgments, the review also questioned the ethical judgments embodied in its definition of risk, noting that evaluating the "acceptability of nuclear reactors solely on the risk of early fatalities, and latent health effects, and property damage for Class 9 [major] accidents is inappropriate. All the issues associated with the nuclear fuel cycle are important, including economic and environmental matters, and weapons proliferation" [(20) p. 40]. Failure to recognize these limits may have

contributed to "instances in which WASH-1400 has been misused...to judge the acceptability of reactor risks" [(20), p. x], thereby neglecting the full risk, costs, and benefits of generating electricity with nuclear power and alternative technologies.

Advances in the theory and application of probabilistic risk analysis can be found in any issue of *Risk Analysis*, *Reliability Engineering and System Safety*, *IEEE Transactions on Reliability*, and related journals. All forms of analysis have the same logic: Decompose complex systems into manageable components and then calculate how they might perform together. All require scientific judgment to bound the set of components and assess the limits to those bounds. All require ethical judgment to determine which outcomes to predict and to extract the policy implications of the results.

Examples of the progress possible when assumptions are clearly documented can be found in the peer-reviewed assessments of microbial risks in a recent issue of *Risk Analysis*. Results there include the possibility of treating municipal wastewater well enough to use for irrigating vegetables (29) and the impossibility of treating victims as a way to eliminate the parasite responsible for schistosomiasis (30). Clear documentation can also reveal fundamental flaws, as when an external review (31) concluded that the Department of Homeland Security's 2006 Bio-terrorism Risk Assessment (32) "*should not be used*" (italics in original), given such problems as using estimates "not supported by any existing data," omitting "economic loss and environmental and agricultural effects," and lacking "a realistic representation of the behavior of an intelligent adversary." The review notes that, for any revision to be useful to decision-makers, "documentation should be sufficient for scientific peer review" [(31), pp. 3–5].

Analyzing risks from multiple sources: Priority setting

The Reactor Safety Study made an explicit ethical judgment in omitting risks related to nuclear proliferation (among others). Some such screening of relevant outcomes is required when bounding any analysis—a process that must balance the risk of looking at so many issues that none are understood well against the risk of ignoring issues that would prove important were they to receive proper attention.

The U.S. Environmental Protection Agency addressed this challenge in the late 1980s and early 1990s in a series of risk-ranking exercises conducted with its staff (33), with its Scientific Advisory Board (34), and, eventually, with citizens from many states and regions (35). In these exercises, participants chose the risks (e.g., infectious disease and urban sprawl) and the valued outcomes (e.g., morbidity, mortality, economic development, and, in one case, the Vermont way of life). Analysts then roughly estimated each outcome for each risk, after which participants compared those expected outcomes in order to set priorities for future analysis and action.

Thus, analysts' work was driven by policy-makers' concerns.

The success of risk-ranking depends on the scientific judgment involved in identifying potentially relevant outcomes and providing the initial estimates. How much experts know about each topic depends on the state of the science. The usefulness of their knowledge depends on how well they can translate it into the terms that decision-makers need. Since the Reactor Safety Study, collaborations between behavioral and decision scientists have developed procedures for expert elicitation (6–10), designed to structure that translation process so as to reduce judgmental biases such as overconfidence and anchoring (36, 37). For example, to avoid the ambiguity of verbal quantifiers (e.g., "common" side effect, "small" risk) (38), these procedures elicit numerical expressions (e.g., "there is a 95% chance of between 5 and 50 people dying in coal mining accidents next year") (39). Expert elicitation has been used in domains as diverse as ocean acidification (40), nuclear power (41), and genetically modified crops (42).

The success of risk-ranking also depends on the ethical judgments made in defining its key terms (3–5, 43). For example, "risk of death" could be defined as "probability of premature mortality" or "expected life-years lost." The former definition treats all deaths as equal, whereas the latter gives extra weight to deaths of young people (who lose more years of expected life) (43, 44). In the United States, similar numbers of people die annually from accidents and chronic lower respiratory diseases (45). By the first definition, the two risks are similar; by the second, accidents are a greater threat (because they disproportionately affect younger people).

One could distinguish among deaths for other reasons, too. For example, Starr's seminal article on risk analysis (46) proposed that people treat voluntary and involuntary risks differently (e.g., skiing versus electric power). Therefore, voluntariness should be part of the definition of risk. Subsequent research has identified other features that might matter to people, such as how equitably risks are distributed (e.g., over people and over time), how well risks are understood (by scientists and by those exposed to the risks), and how much dread (and psychological discomfort) risks evoke (5, 46–48).

Understanding Risk, an influential National Research Council (49) report, proposed an analytical-deliberative process for defining "risk," thereby deciding which features to consider and ignore. In that process, stakeholders state their concerns. Analysts then propose a formal expression that is precise enough to be used in quantitative analyses. Stakeholders deliberate that proposal in terms of whether it captures their intent, iterating as needed with the analysts, in the manner of EPA's risk-ranking exercises.

Having stakeholders define the terms of an analysis tailors it to their needs. However, it also limits comparisons across analyses, if each has its own definition of "risk" (or "cost" or "benefit")

(50). Figure 1 shows a standard definition protocol endorsed by the U.K. government's economic and finance ministry (51). On the left are two outcomes that might be monetized as how much people should be willing to pay to eliminate them. On the right are six societal concerns, representing reasons people might view risks with otherwise similar outcomes differently (3–5, 46–49). The format invites discussion of whether concerns that affect feelings should also affect policies (e.g., “I dislike risks that are unfamiliar to me, but what matters is how well scientists understand them”) (52). The format also legitimates asking about the importance of nonmonetary concerns (e.g., are death and harm worse when equity is violated, in the sense that the people who bear the risk are not those who stand to benefit?) This protocol was illustrated in the context of whether to treat all roadway accidents similarly or, for example, to give added weight to those affecting children (51).

The success of an analytical-deliberative process is an empirical question (11, 12, 53, 54). One measure of that success is how well the process enables participants to understand the risks and develop stable preferences among them (e.g., can they make sound inferences based on what they know? Do their preferences change when additional perspectives are suggested?). A second measure is whether a process leads to fewer, but better, conflicts, by focusing participants on genuine disagreements and avoiding ones that arise from misunderstanding (e.g., can participants describe their opponents' position, even when they reject it?). Rather than seeking consensus, these processes accept the legitimacy of informed differences and try to articulate their policy implications. For practical purposes, it might be enough for participants to agree about which risks rank highest, and hence deserve attention,

and which rank lowest, and hence can be set aside (55). If systematic prioritization fails these tests, then decision-makers might be better off “muddling through,” in the sense of tackling problems as they arise and reshaping their priorities as they go along (56–58). Proponents of deliberative democracy (59) study how such focused processes compare to conventional (“aggregative”) policy-making. For example, the Energy Systems Project was a national consultation that found perhaps surprising agreement in its diverse participants' visions for U.K. energy futures (60).

Analyzing risks per unit of benefit

Risk decisions are rarely about risks alone. Large risks may be acceptable if they bring large benefits and there are no good ways to reduce them. Small risks may be unacceptable if they bring small benefits or could be easily reduced (3, 43, 44, 61).

Thus, making sound decisions means comparing the expected risks, costs, and benefits of the available options. In an early step toward informing choices among ways to generate electricity, Inhaber (62) estimated their “risk per megawatt-year of electric power.” He defined “risk” as the number of workdays lost from injury and the number of deaths, treating all deaths as equal. Predictably, given the stakes, his work met vigorous criticism on both ethical and scientific grounds (63) and was followed by more ambitious analyses (64–66).

Such calculations are special cases of life-cycle analysis, which tries to account for all of the energy and materials involved with creating, using, and disposing of products, processes, or services (67, 68). That accounting depends on the bounds of the analysis, including how far it goes upstream (e.g., does it consider the energy and material embodied in equipment?) and down-

stream (e.g., does it include methane releases from landfills?). Setting those bounds requires scientific judgments (e.g., is the risk from terrorist attacks large enough to include in the analysis?) and ethical ones (e.g., do effects in other countries matter?).

Once the bounds are set and expected outcomes estimated within them, decision-makers must weigh those outcomes against one another. Cost-benefit analysis (69) does that by translating all outcomes into a common unit: money. In the United States, cost-benefit analysis got one push from a mandate to monetize the effects of water projects (70) and another from President Reagan's Executive Order 12291 (71) requiring analysis of the “net benefit to society” of “major rules” and “alternative approaches that could substantially achieve the same regulatory goal.”

As an example of such analyses (and their assumptions), economists from the U.S. Environmental Protection Agency in 2014 contrasted the costs of compliance estimated before and after implementing various regulatory rules (e.g., the 2001 National Primary Drinking Water Regulations for Arsenic and the 1998 Locomotive Emission Standards) (72, 73). Observing that the actual costs were generally less than the predicted ones, these analysts attributed that bias to innovative cost-cutting (spurred by the regulations), incomplete compliance, and initial estimates based on industry data that deliberately overestimated anticipated costs. They also lamented the inconsistent bounds that complicated comparing analyses (e.g., some considered just capital costs, whereas others included both capital and operating costs).

Market prices provide one source of guidance for monetization, whose interpretation requires both scientific and ethical judgments. For example, car prices reflect their benefit to buyers. If a buyer gets a bargain, then there is consumer surplus, and the price is less than the car's worth. If a market suffers from monopoly pricing, misleading advertising, or questionable loan practices, then a car's price might not capture its worth (or full cost). In such cases, using that price might bias estimates (a question of science) or endorse unfair practices (a question of ethics).

For outcomes that are not traded in efficient markets, other measures of value are possible. For example, tax credits (e.g., for fuel efficiency) might be taken as capturing benefits to society beyond those received by consumers. If the bounds of an analysis include the externalized costs of driving (i.e., those imposed on others), then those costs might be partially captured by hospital bills for lung disease (due to particulate matter) and aspirin sales (due to tropospheric ozone) (69, 70). The travel-cost method monetizes destinations by what people pay to see them (74, 75). Thus, the benefits of wilderness areas are partially captured by visitors' entry fees, fuel and lodging costs, and outfitting expenses. The cost of preserving those areas might be partially captured by the economic benefits promised by would-be developers. One tongue-in-cheek analysis applies travel-cost method assumptions made in one analysis (the Roskill

Concern assessment framework

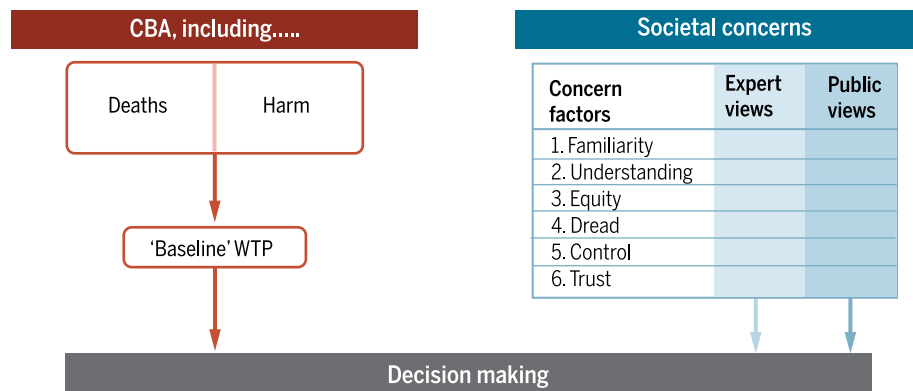


Fig. 1. A standard method for defining risk. The attributes on the left are calculated according to procedures from cost-benefit analysis (CBA), producing a “baseline” amount that society should be willing to pay (WTP) to eliminate a risk. The attributes on the right are assessed by judgments, with five levels meant to apply across hazards. For Equity, those levels are (i) harms and rewards are distributed fairly; (ii) some suffer more than others but receive additional compensation; (iii) benefits are distributed fairly, harms are distributed unfairly; (iv) harms and rewards are both distributed unfairly; and (v) a small minority benefits to the extreme detriment of all others. [Source: (3) by permission of Oxford University Press]

Commission) to justify leveling Westminster in favor of a third London airport, given the time that travelers would save in getting to such a central location (76). Ecosystem services analyses assess the economic benefits of natural systems, such as the flood protection provided by marshes and barrier islands (77).

Human life, a focus of many risk analyses, is, of course, not traded directly in any legitimate marketplace. One possible way to monetize the value of human life is in terms of wage premiums for riskier jobs (e.g., receiving \$X for assuming a Y% increase in premature death) (78–80). Such analyses require scientific judgments in order to hold constant factors that can make entry-level jobs riskier than better-paid senior ones and the ethical judgment of accepting wages as measuring individuals’ worth (81). A common value in current U.S. regulatory analyses is \$6 to 7 million per life. Considering lost life-years (and not lost lives) has been criticized on ethical grounds (51), sometimes as applying a “senior discount” (82).

These approaches analyze the preferences “revealed” in market transactions. In the absence of such transactions, stated preference methods ask people how much they are willing to pay to gain an outcome [or, less commonly, how much they are willing to accept for losing it (83)]. One variant, the contingent valuation method, asks respondents to imagine a market offering a transaction that links money to a valued outcome (e.g., the opportunity to pay to preserve an endangered species) (84). Its use in monetizing damages from the Exxon Valdez grounding sparked lively debate (85, 86).

One recurrent topic for stated preference methods is whether to use surveys, which ask people to answer questions on their own, or interactive methods, where moderators help people think through their answers, as with risk-ranking or decision analysis (35, 87). Surveys produce biased estimates when respondents fail to think of all relevant perspectives by themselves. Interactive methods produce biased estimates when moderators fail to present all perspectives fairly (11, 12, 35, 87–89). A second recurrent topic is how people deal with missing details. For example, if a question says nothing about distributional effects, do respondents try to guess what those might be,

feel implicit pressure to ignore them, or resent the implication that equity does not matter?

Analyzing risks and benefits: Evaluating medical treatments

When the U.S. Food and Drug Administration (FDA) decides whether to approve drugs, the diverse, uncertain benefits and risks defy monetization. Nonetheless, manufacturers still want predictability in FDA’s rulings. Recognizing that desire, FDA has committed to developing a more explicit form of analysis than its traditional reliance on expert judgment, whereby its reviewers consult with one another and FDA advisory panels before rendering a narrative summary of their conclusions (90).

One potential approach to greater predictability is cost-effectiveness analysis, developed to allocate resources among approved medical treatments (91). It typically measures expected health benefits in quality-adjusted life-years (QALYs) or similar units (92, 93). Because those benefits represent reduced risks (of ill health), measuring them faces the same issues as measuring “risk.” Ethically, looking at years of life saved should be uncontroversial: More years are better. However, considering the quality of those years means placing less value on years of ill health (and, perhaps implicitly, on individuals experiencing them). Scientifically, analysts must judge how well people can answer QALY questions such as “How many years of perfect health would be equivalent to 10 years with limited mobility?”

With any stated preference method, respondents must first understand the outcomes (how mobile will I be?), then imagine the subjective experience (how will I tolerate the lack of mobility?), and finally translate those feelings into allowable answers (equivalent years of perfect health). Evaluating respondents’ success requires scientific judgment of the construct validity of their responses. That is, to what extent are they sensitive to relevant features of questions (e.g., whether the cure rate is 30% or 50%) (94) and insensitive to irrelevant ones (e.g., whether the question asks about the probability of death or the complementary probability of survival) (95, 96).

Although QALY-like approaches have strong advocates (97, 98), FDA has adopted a benefit-risk framework (Fig. 2) that does not translate all

outcomes into a common unit but leaves them in their natural units (e.g., survival rates and mobility). The left-hand side of Fig. 2 summarizes FDA’s analysis of the evidence (99). The right-hand side interprets those findings in terms of FDA’s regulatory mandate. The bottom box explains FDA’s weighing of the expected risks and benefits, given the nature of the medical condition, the unmet medical need (with other treatments), and the plans for managing residual risks if the product is approved. The top row of the framework addresses a scientific and ethical question facing any analysis that makes choices on others’ behalf: Do the revealed or stated preferences used in the analysis adequately represent those individuals’ concerns? “Analysis of condition” is meant to convey a feeling for, say, life with the psychological sequelae of sickle cell disease or the incapacitating constipation that can accompany irritable bowel syndrome. FDA’s Voice of the Patient initiative (100) seeks to inform that summary by hearing directly from patients and advocates.

Preserving the reality of patients’ experience is one reason for leaving estimated risks and benefits in their natural units, rather than using standard measures of benefit and risk (e.g., QALYs). Those estimates, along with the narrative explaining FDA’s approval decision, preserve some of the richness in its reviewers’ intense deliberations over the evidence. A second reason for not using standard measures is that FDA recognizes that patients’ preferences may differ. As a result, it provides them with the inputs needed to make personally relevant choices. How people make such difficult trade-offs (e.g., when drugs offer both benefits and risks) is an active area of research (88, 89, 94–96, 101).

Thus, instead of a computed weighting of risks and benefits expressed in a common unit, FDA offers a narrative weighing of estimated outcomes. As a result, FDA’s regulatory decisions are less predictable than they would be were FDA bound by a calculation. However, if the retained richness of the summaries helps FDA to construct more stable preferences and to explain them more fully, then more predictable policies should reveal themselves over time, without calculations (102, 103).

Communication and coordination

Sound analysis requires sound communication between the analysts and the stakeholders whom they serve. Analysts need to know which issues matter to stakeholders so that they can make appropriate ethical judgments when bounding analyses and defining their terms. Stakeholders need to know what scientific judgments analysts made when conducting analyses so that they can decide how much to rely on their conclusions.

Given the social, professional, and institutional distance between most analysts and stakeholders, such communication requires deliberate coordination, with a structured process like that in Fig. 3 (104), whose features echo those of many related proposals (11, 12, 35, 48–54, 105, 106). Its horizontal arrows imply continuing engagement, offering stakeholders an opportunity to hear and be heard,

Decision factor	Evidence and uncertainties	Conclusions and reasons
Analysis of condition		
Current treatment options		
Benefit		
Risk		
Risk management		
Benefit-Risk summary assessment		

Fig. 2. FDA’s Benefit-Risk Framework, summarizing its interpretation of the evidence relevant to its decision to approve a pharmaceutical. [Source: (90)]

at each stage of the process. The vertical arrows imply continuing reflection by analysts on the progress of the work, with the opportunity and obligation to decide whether to continue, revise, or abandon it.

Among the examples above, EPA's risk-ranking exercises come closest to such a process, whereas Inhaber's analysis of the risks of generating electricity is the furthest away, guided solely by his intuitions about public concerns. Although stakeholder input was not part of WASH-1400, the recent Blue Ribbon Commission on American's Nuclear Future called for proactive engagement (106). FDA's framework was developed for use by its staff to protect the confidentiality of clinical trial data. However, its implementation requires stakeholder input.

Thus, the social context of analyses varies and, with it, the collaborative process that is needed and possible. Within those constraints, the science of stakeholder participation can provide guidance (11, 12, 107). It emphasizes creating respectful relationships, whereby stakeholders need not struggle to hear or be heard. That science has evolved from a simplistic Decide-Announce-Defend model, delivering analysts' conclusions, to sustained, two-way communications, with stakeholders helping to define and interpret analyses. Recent examples of such processes include consultations over British energy policy (60), Cultus Lake (British Columbia) salmon (17), and the development of Zurich North (108),

The science of science communication provides guidance on how to create the content for such processes (109–117). Research here began with (what is now called) a “deficit model,” whereby experts decide what knowledge people need to be deemed “literate” (about science, energy, climate, finance, and so on). The research has evolved to user-centered models, which begin by analyzing the decisions facing stakeholders, in order to identify the few things that they most need to know from among all those things that it might be nice to know. The work then proceeds by drafting messages designed to close critical gaps, testing their efficacy, and repeating as necessary until people are adequately informed.

Applying the science of communication to the communication of science requires overcoming three common barriers. One is the natural tendency for people to overestimate how well they know what other people are thinking and vice versa (112). As a result, people fail to learn enough about their audience to communicate effectively—and then may blame the audience when their message inexplicably does not get through. A second barrier is casual analysis of stakeholders' information needs. For example, different facts about storm surges may be critical for the same individual when deciding whether to evacuate, buy a home, or support zoning rules. It takes analysis to determine the facts that people facing each decision need (39, 110, 113). A third barrier to effective communication is many experts' conviction that lay people cannot grasp technical information. So why bother trying? The limits to

lay judgments are, indeed, well documented (22, 36, 37, 47). However, that research also identifies ways to address these limits (39, 107–114). Moreover, for securing public trust, even poor communication may be better than silence, by showing respect for the public's right to know, even when meeting it clumsily (4, 11, 12, 48, 53).

Two-way communication might increase the cost of analysis. However, it can also increase its expected benefit by forestalling the radical skepticism that can poison public discourse once science loses public trust (115–118). At the extreme, stakeholders may reject analysis *per se*, as when they advocate precautionary principles that proscribe highly uncertain actions with great potential risks (119, 120). These principles reject a tenet of rational analysis, namely, that any risk can be acceptable, given enough compensating benefit. By offering simple decision rules, precautionary principles may also provide a way to address a seeming imbalance of power, when stakeholders feel excluded from setting the terms of an analysis or without the resources to check its calculations (4, 28).

Conclusion: Skill and wisdom in analysis

The science of analysis has seen advances in both the sophistication of its calculations and the awareness of the ethical and scientific judgments that they entail. It has also developed better ways to integrate behavioral science knowledge when

communicating with stakeholders, eliciting expert knowledge, assessing preferences, and anticipating the effects of behavior (e.g., of operators or patients). Often, these advances emerged from controversies that revealed limits to analytical conventions, such as how problems are bounded or “risk” is defined. In other cases, the advances emerged from analysts grappling with the unique properties of new problems, as might be expected in future work analyzing the expected effects of hydrofracking, nanobeads, robotic surgery, commercial drones, or gain-of-function research on pathogens with pandemic potential.

To realize the potential of their science, analysts must create partnerships with the stakeholders who depend on their work, so that analyses are relevant and understood. To that end, analysts' professional standards should require full disclosure of the scientific and ethical judgments made in formulating and executing analyses. However, those disclosures may not satisfy stakeholders unless preceded by continuing engagement with them. Together, analysts and stakeholders need to determine how best to invest analytical resources, resolve definitional issues, and interpret the resulting estimates. Through interactions like those depicted in Fig. 3, analysts can share their expertise while creating the mutually respectful personal relations needed to secure a trusted hearing for their work.

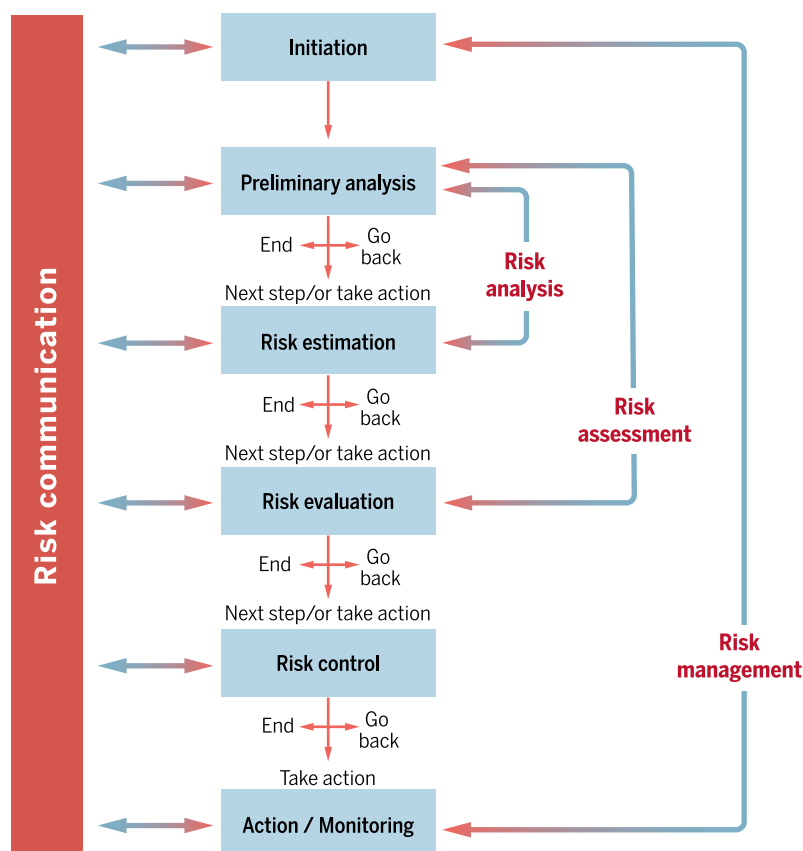


Fig. 3. A process for ensuring ongoing communication between analysts and stakeholders.
[Source: Adapted from (104)]

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RESEARCH ARTICLE SUMMARY

PRIMATE EVOLUTION

Miocene small-bodied ape from Eurasia sheds light on hominoid evolution

David M. Alba,* Sergio Almécija, Daniel DeMiguel, Josep Fortuny, Miriam Pérez de los Ríos, Marta Pina, Josep M. Robles, Salvador Moyà-Solà

INTRODUCTION: Reconstructing the ancestral morphotype from which extant hominoids (apes and humans) evolved is complicated by the mosaic nature of ape evolution, the confounding effects of independently evolved features (homoplasy), and the virtual lack of hylobatids (gibbons and siamangs) in the Miocene fossil record. For several decades, small-bodied anthropoid primates from Africa and Eurasia have not played an important role in this debate, because they generally lack the shared derived features of extant catarrhines (hominoids and Old World monkeys) and are thus considered to precede their divergence. Even some small-bodied catarrhines from Africa (dendropithecids), considered to be stem hominoids by some authors, are viewed as more

primitive than the larger-bodied stem ape *Proconsul*. This has led to the assumption that hylobatids are a dwarfed lineage that evolved from a larger-bodied and more great ape-like common ancestor with hominids (great apes and humans).

RATIONALE: Here we describe a new genus of small-bodied (4 to 5 kg) ape from the Miocene (11.6 Ma), discovered in the Abocador de Can Mata stratigraphic series (Vallès-Penedès Basin, northeast Iberian Peninsula), that challenges current views on the last common ancestor of extant hominoids. This genus is based on a partial skeleton that enables a reliable reconstruction of cranial morphology and a detailed assessment of elbow and

wrist anatomy. It exhibits a mosaic of primitive (stem catarrhine-like) and derived (extant hominoid-like) features that forces us to re-evaluate the role played by small-bodied catarrhines in ape evolution.

RESULTS: The new genus retains some features that are suggestive of generalized above-branch quadrupedalism, but it possesses more extensive hominoid-like postcranial features (mostly related to enhanced forearm rotation and ulnar deviation capabilities) than those convergently displayed by atelids. Its overall body plan is more compatible with an emphasis on cautious and eclectic climbing, combined with some degree of below-branch forelimb-dominated suspension (although less acrobatic than in extant gibbons). Its relative brain

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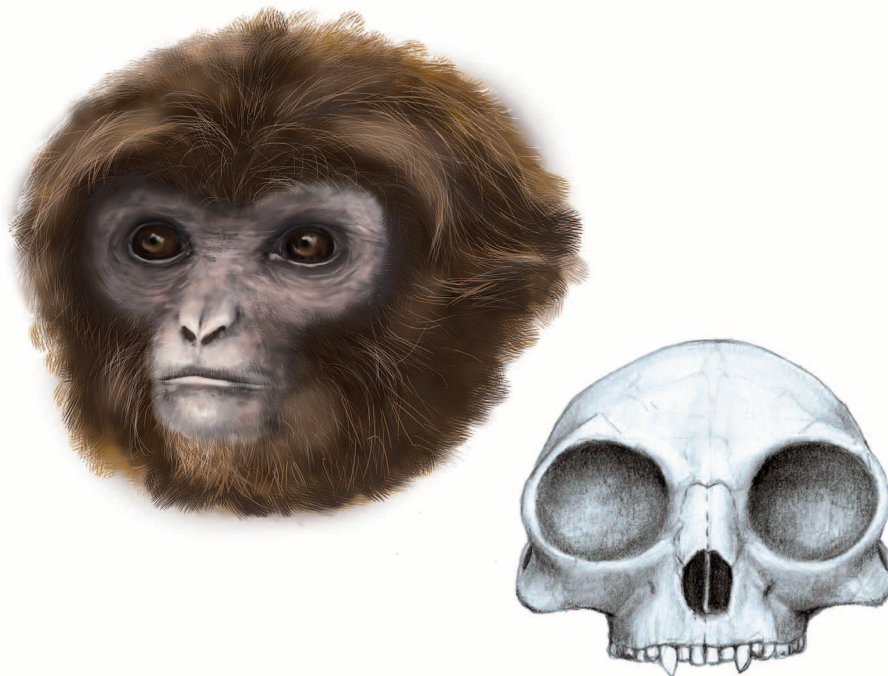
size implies a monkey-like degree of encephalization (similar to that of hylobatids but below that of great apes), and dental microwear indicates a frugivorous diet. From a phylo-

genetic viewpoint, the new genus combines craniodental and postcranial primitive features (similar to those of dendropithecids) with multiple derived cranial and postcranial features shared with extant hominoids. Some cranial similarities with gibbons would support a closer phylogenetic link between the new genus and hylobatids. However, this possibility is not supported by the total evidence. A cladistic analysis based on more than 300 craniodental and postcranial features reveals that the new genus is a stem hominoid (preceding the divergence between hylobatids and hominids), although more derived than previously known small catarrhines and *Proconsul*.

CONCLUSION: As the first known Miocene small-bodied catarrhine to share abundant derived features with extant hominoids, the new genus indicates a greater morphological diversity than previously recognized among this heterogeneous group, and it provides key insight into the last common ancestor of hylobatids and hominids. Our cladistic results, coupled with the chronology and location of the new genus, suggest that it represents a late-surviving offshoot of a small African stem hominoid that is more closely related to crown hominoids than *Proconsul* is. These results suggest that, at least in size and cranial morphology, the last common ancestor of extant hominoids might have been more gibbon-like (less great ape-like) than generally assumed. ■

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Cranial reconstruction and life appearance. Artist's representation of the cranial reconstruction (in frontal view) and of the life appearance (in lateral oblique view) of the new genus of small-bodied ape from the Iberian Miocene. [Artwork by M. Palmero]

RESEARCH ARTICLE

PRIMATE EVOLUTION

Miocene small-bodied ape from Eurasia sheds light on hominoid evolution

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Miocene small-bodied anthropoid primates from Africa and Eurasia are generally considered to precede the divergence between the two groups of extant catarrhines—hominoids (apes and humans) and Old World monkeys—and are thus viewed as more primitive than the stem ape *Proconsul*. Here we describe *Pliobates cataloniae* gen. et sp. nov., a small-bodied (4 to 5 kilograms) primate from the Iberian Miocene (11.6 million years ago) that displays a mosaic of primitive characteristics coupled with multiple cranial and postcranial shared derived features of extant hominoids. Our cladistic analyses show that *Pliobates* is a stem hominoid that is more derived than previously described small catarrhines and *Proconsul*. This forces us to reevaluate the role played by small-bodied catarrhines in ape evolution and provides key insight into the last common ancestor of hylobatids (gibbons) and hominids (great apes and humans).

Apes and humans (hominoids) diverged from Old World monkeys (cercopithecoids) by the Oligocene [≥ 25 million years ago (Ma)] (1, 2) and subsequently diversified in both Africa and Eurasia during the Miocene (23 to ~5 Ma) (3). They are currently represented by crown hominoids (4)—i.e., the small-bodied hylobatids (gibbons and siamangs) and the larger-bodied hominids (great apes and humans), which diverged from one another by the early Miocene (~17 Ma) (7). Reconstructing the ancestral morphotype from which extant apes and humans evolved is a challenging task (5–7), given the mosaic nature of evolution (8), the confounding effects of independently evolved features (homoplasy) (5, 9), the conflicting evidence provided by Miocene great apes (6, 9), and the incomplete and fragmentary nature of the hominoid fossil record [in particular, the virtual lack of fossil gibbons until at least the latest Miocene (10, 11)]. Thus, although extant hominoids share numerous derived features, particularly in the trunk and forelimb, it is uncertain to what extent these characteristics were inherited from their last common ancestor, whose morphotype is still under discussion (5, 6, 11, 12).

The earliest hypotheses postulated a small-bodied gibbon-like ancestor (13), and for many

years, fossil small-bodied catarrhines (anthropoid primates from Africa and Eurasia) were considered to be broadly ancestral to gibbons (14, 15). Today, hylobatids are generally considered to be a specialized and probably dwarfed lineage, evolved from a larger and more great ape-like last common ancestor with hominids (6, 16). This is because known small-bodied extinct catarrhines, such as the African dendropithecids (including at least *Dendropithecus*, *Simiolus*, and *Micropithecus*) and the Eurasian pliopithecoids (*Pliopithecus*, *Epipliopithecus*, and allied genera), lack most of the synapomorphies (shared derived features) of crown catarrhines (17–21). Even dendropithecids, which are more derived than pliopithecoids and are currently interpreted by some authors as stem hominoids (preceding the hylobatid-hominid divergence) (3, 8, 22), are considered to be more basal than the stem ape *Proconsul* (8, 21–23). Here we describe a new Miocene small-bodied ape from Spain, *Pliobates cataloniae* gen. et sp. nov. (24). In some primitive features, this new primate resembles previously known small-bodied catarrhines such as dendropithecids, but it differs from them and from *Proconsul* by displaying multiple crown-hominoid derived features. This mosaic provides key insight into ape evolution by forcing us to reevaluate the role played by small-bodied catarrhines in the emergence of crown hominoids.

Provenance

The new taxon is described on the basis of a partial skeleton (IPS58443; table S1) that is permanently housed in the collections of the Institut Català de Paleontologia Miquel Crusafont (ICP) in Sabadell, Spain. The main cranial fragments were initially discovered on 3 January 2011 by a team of paleontologists from the company FOSSILIA Serveis Paleontològics i Geològics, directed by one of the authors (J.M.R.), while overseeing the

excavation works performed by digging machines at the Can Mata landfill (els Hostalets de Pierola, Catalonia, Spain). In the following days, postcranial elements were found at the field during excavation of the same stratigraphic level, and the remaining postcranial small bones and other small fragments were subsequently recovered by screen-washing the excavated sediments. All the fossils therefore come from a single horizon, which was labeled ACM/C8-A4 (Abocador de Can Mata, Cell 8, sector A, locality 4).

The ACM local stratigraphic composite series (25–27) is located in the Vallès-Penedès Basin (northeast Iberian Peninsula), a half-graben trending north-northeast–south-southwest and limited by the Catalan Coastal Ranges; this structure was generated by the rifting of the northwest Mediterranean during the Neogene (28, 29). The basin infill mostly consists of marginal alluvial fan sediments that have provided a rich fossil record of Miocene terrestrial vertebrates (30). The area of els Hostalets de Pierola has thick middle to late Miocene sequences that were deposited in distal-to-marginal inter-fan zones of coalescing alluvial fan systems (27). The ACM composite series, about 250 m in thickness, includes more than 200 formally defined localities that can be accurately dated based on lithostratigraphic, magnetostratigraphic, and biostratigraphic correlation (26, 27, 31). The whole series is late Aragonian, and, based on updated chron boundaries (32), it spans from ~12.6 to 11.5 Ma. Locality ACM/C8-A4 is correlated to chron C5r.2n (11.657 to 11.592 Ma) with an interpolated age of 11.628 Ma, which is close to the middle/late Miocene boundary, as defined by the base of the Tortonian and currently dated to 11.625 Ma (32).

Methods
Cranial reconstruction

The cranium was preserved in a main piece (IPS58443.1) with parts of the neurocranium, basicranium, muzzle, and right maxilla (Fig. 1, A to C); a medium-sized fragment with the left maxilla (IPS58443.2); and other smaller fragments that were found in close spatial association or recovered by screen-washing the surrounding sediments. These other fragments include part of the occipital and right parietal (IPS58443.3), the right glenoid region (IPS58443.4), the right occipital condyle (IPS58443.5), a fragment of the right orbital margin (IPS58443.6), the left orbital margin and temporal process of the zygomatic (IPS58443.12), a parietal fragment (IPS58443.11), and several other minor fragments whose location cannot be determined (IPS58443.7 to IPS58443.10, IPS58443.13, and IPS58443.37). The mandible was not preserved, except for a fragment of the right ramus with the condyle (IPS58443.14). The main fragment (IPS58443.1) is composed of several bone fragments that are crushed against each other and somewhat displaced from their anatomical position, but (like the remaining specimens) not plastically distorted.

Several bone fragments of IPS58443.1 were individualized through careful mechanical preparation, but for many other bone fragments, their

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fragility and state of preservation precluded a complete isolation from each other and/or from the embedding matrix. Therefore, for conservation reasons, no complete preparation of the specimen was performed, and a virtual three-dimensional (3D) reconstruction was undertaken instead. The larger specimens (IPS58443.1 and IPS58443.2) were scanned at the American Museum of Natural History (New York) with a high-resolution computed tomograph (CT) system (Phoenix v|tome|x s180, General Electric Measurement & Control Solutions, Hanover, Germany), using a nanofocus x-ray tube. Different protocols were used for these two cranial fragments: 160 kV voltage, 1.4 mA current, 0.2 mm Cu filter, and magnification of 2.10013075, obtaining 1600 slices (virtual cross-sectional images) of 0.2 mm in thickness and a pixel size of 0.09523217 mm (IPS58443.1); and 145 kV voltage, 1.3 mA current, 0.1 mm Cu filter, and a magnification of 2.93159482, obtaining 1500 slices of 0.2 mm in thickness and a pixel size of 0.06822225 mm (IPS58443.2). Raw

CT data were imported into VGStudio Max 2.1 and exported (as a stack of TIFF files) to Avizo 7.0 and Geomagic 2012 for segmentation, repositioning, mirroring, and/or visualization. CT-scanned bone fragments were segmented slice by slice by digitally removing the matrix with the aid of differential bone and sediment densities, using the semiautomatic thresholding tools of Avizo 7.0. Additional small isolated bone fragments not surrounded by matrix were scanned with a 3D desktop laser scanner (NextEngine) at high definition and with a dimensional accuracy of 0.13 mm (Macro Mode); the resulting 3D models were exported to Geomagic 2012 to align and repair the meshes. The preserved bone fragments generally had clean fractures that allowed them to be easily matched with other fragments. Up to 39 3D virtual models of bone fragments, including pieces of the premaxillae and maxillae with teeth, lacrimals, zygomatics, frontal, parietals, temporals, occipital, and pterygoids, were thus digitally assembled and repositioned using Avizo 7.0 and Geomagic 2012 on

the basis of preserved morphology, fracture congruence, and bilateral symmetry. Once the preserved fragments were adequately positioned, areas preserved only in one side of the cranium were mirrored. The individual models were then merged to obtain the definitive 3D virtual model, which was also 3D-printed with a ZPrinter 450 at the Universitat Autònoma de Barcelona for visualization and comparative purposes.

To reconstruct the muzzle area, we primarily relied on IPS58443.1, in which the right premaxilla (with the alveoli for I1 to C1) and the maxilla (with P3 to M3, the lower portion of the nasal aperture, most of the palatine process, and the intermaxillary suture) are almost completely preserved. The left maxillary specimen (IPS58443.2) is less complete and only includes the M2 to M3 series and a smaller portion of the intermaxillary suture, as well as the zygomatic root. Palate width and shape were thus reconstructed by mirroring the right fragment onto the left one, then further adjusting it based on the fit onto the left portion of the intermaxillary suture and the alignment with the left molars. With regard to the orbital area, although the orbits are not completely preserved on either side, their profile can be reconstructed with reasonable accuracy based on the available fragments: IPS58443.1 includes, in several fragments, most of the interorbital area of the frontal and the superior orbital rims, the maxillary portion of the right inferior orbital rim, a zygomatic portion of the right lateral orbital rim, and both lacrimals; IPS58443.12 includes a larger lateral portion of the left orbit that encompasses part of the temporal process of the zygomatic arch and the frontomaxillary suture. Although the zygomaticomaxillary suture is not preserved on either side, the missing portion of the zygomatic process of the maxilla on the left side is minimal. Therefore, it is possible to situate the left orbit relative to the muzzle, on the basis of the orientation between the zygomatic process of IPS58443.12 and the zygomatic root preserved in IPS58443.2. Similarly, although the frontozygomatic suture is not preserved at the end of the left zygomatic process of IPS58443.1, only a very small portion is missing; thus, the preservation of the suture in the zygomatic portion of the lateral orbital rim in IPS58443.12 enables a reliable reconstruction of the superolateral aspect of the orbit, based on the curvature of these two fragments. The inferior orbital rim can be also reconstructed using its right maxillary portion, which is preserved in IPS58443.1, by mirroring onto the left side. Moreover, the preservation of both superior orbital rims in the frontal fragments of IPS58443.1 further allows the right orbit to be positioned relative to the muzzle, in spite of the fact that the right zygomaticomaxillary suture is not preserved. Overall, although the missing fragments of the orbital rims introduce some degree of uncertainty, the curvature of the various preserved portions enables a confident reconstruction of orbital size and shape. The preserved portions of the zygomatic and maxilla further enable reliable positioning of the orbits relative to the muzzle. Because the missing portions

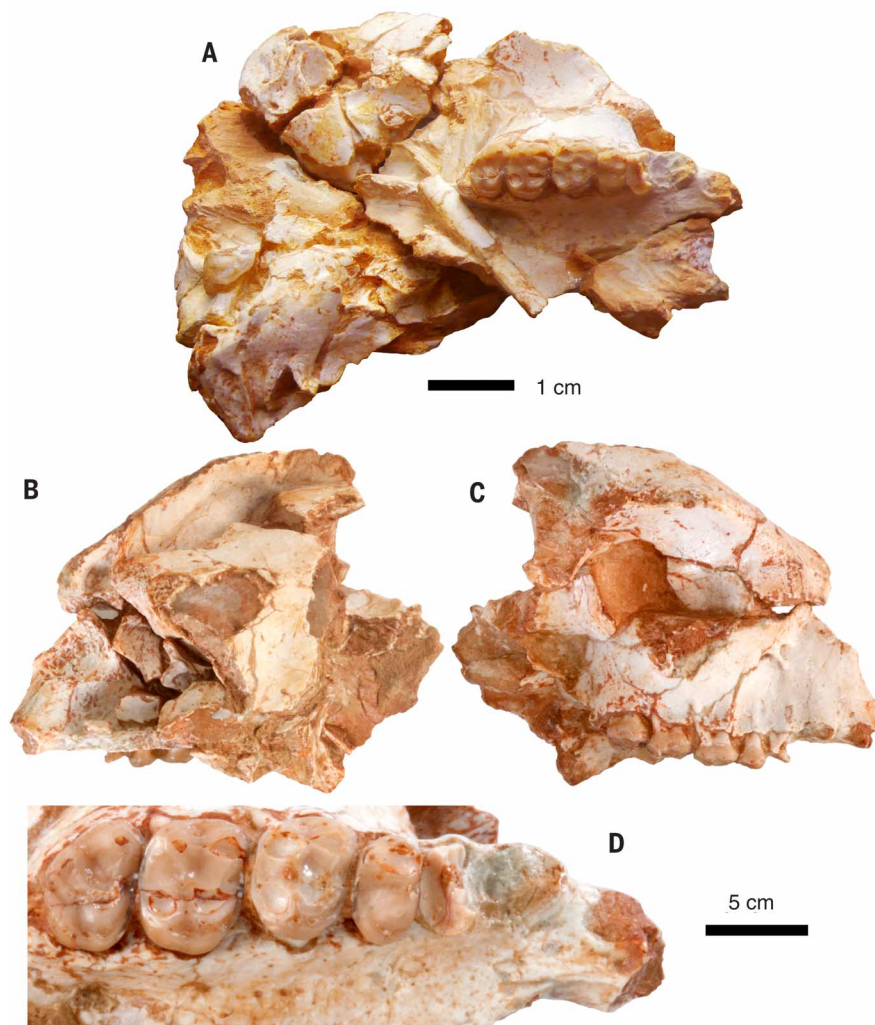


Fig. 1. Cranium and dentition. (A to C) Cranium of the holotype (IPS58443) of *Pliobates cataloniae* gen. et sp. nov. The main cranial fragments, including the basicranium and the right palate, are shown in basal view (A); details of the right palatal fragment are shown in left-lateral (B) and right-lateral (C) views. (D) Detail of the right postcanine teeth, in occlusal view (mesial is to the right).

are very small, other possible reconstructions do not differ substantially from the one provided here.

To reconstruct the neurocranium, we relied on the relative spatial position of the braincase fragments in the crushed main specimen (IPS58443.1). Because these fragments are not plastically deformed, they show the original curvature of the braincase, which considerably facilitated the reconstruction of its overall shape. The frontals are preserved in six fragments that enable the reconstruction of most of the braincase morphology (including the whole supraorbital area and the connection with the parietals on the left side). The parietals are preserved in 19 fragments, which have well-preserved sutures and neat fractures. The latter fact, together with the spatial association among the various fragments, enables the reconstruction of the posterior contour of the cranial vault. Large portions of both temporals, including the tympanic bullae, are also preserved in IPS58443.1. The right temporal is more completely preserved than the left one; it includes not only the glenoid fossa and the temporal process of the zygomatic arch, but also the suture with the parietal. These preserved pieces, mirrored onto the left side, enabled the reconstruction of the lateral aspects of the neurocranium. The occipitals are less completely preserved, being restricted to three basioccipital fragments and one occipital fragment, which include the occipital condyles and enable a reliable orientation of the preserved profile of the foramen magnum. One of these fragments connects with the left temporal, thereby enabling the reconstruction of the basicranium. Only minor displacements, caused by diagenesis, had to be corrected; these included displacements between the left bulla and the left occipital condyle and basioccipital, and the artifactual separation of the occipital-temporal suture. Once these elements were slightly repositioned into their correct anatomical place, they fit congruently with the surrounding elements. Last, the pterygoid wings are also preserved on the right side, attached to the palatine but slightly crushed, so that some realignment of this bone was necessary. The connection of the braincase with the superior portion of the face is possible through the frontal fragments, which on the left side are continuous with the parietal fragments. The connection between the braincase and the lower portion of the face is possible, not only because of the preservation of the superior orbital rims in the frontal and of a maxillary portion of the right inferior orbital rim, but also because part of the right pterygoid wing (anatomically adjacent to the palatine) was preserved in its original anatomical position, attached to one of the bone fragments of IPS58443.1 (which includes a large portion of the basicranium and temporal). This pterygoid fragment enables a reliable reconstruction of the relative position of the muzzle and the neurocranium via the basicranium, and it further confirms the correctness of the reconstruction of orbital shape and position. The position of the muzzle was further tested on the basis of the congruent alignment on the right side between the temporal process of the zygomatic in

IPS58443.12 and the zygomatic process of the temporal in IPS58443.4.

Dental measurements and body mass estimation

Standard dental measurements of mesiodistal length (MD) and maximum buccolingual breadth (BL) were taken (in millimeters) to assess dental size and proportions by means of comparative bivariate plots of log-transformed BL versus MD for each of the upper molars. Data for extant and extinct small-bodied catarrhines were taken from the literature (17, 33–46) or measured by one of the authors (D.M.A.). Based on MD and BL measurements, tooth square area (A, in square millimeters) was computed for postcanine teeth to estimate the body mass (BM, in kilograms) of *Pliobates*, using allometric equations of BM versus A (47). BM of *Pliobates* and, for comparative purposes, of *Epipliopithecus vindobonensis* was also estimated based on allometric equations, using postcranial estimators (48) separately for samples of hominoids and catarrhines (i.e., hominoids plus cercopithecoids), based on sex-species mean data. Six postcranial BM estimators were used (49), including three surface areas (for the tibia, humerus, and radius) and three linear measurements (for the humerus and radius; tables S2 and S3).

Humeral torsion and arm angle

Humeral torsion, or the orientation of the humeral head relative to the mediolateral axis of the distal humerus (50), cannot be directly computed in the described skeleton because the humeral head is missing. Accordingly, humeral torsion was kindly estimated by S. Larson, following her methodology (50) for incomplete humeri lacking the proximal end (based on the bisector of the bicipital groove, indicating the orientation of the humeral head), as well as using the posterior buttress for the humeral head (as a reference for the head axis). Measurements were obtained from a cast of the two humeral diaphyseal fragments of the holotype specimen. This humerus was originally preserved as a single specimen, with a crack filled with sediment at about midshaft level, the two fragments being slightly crushed against each other in the proximodistal direction. After manual separation of the matrix, the original shape of the diaphysis was reconstructed by correctly aligning casts of the two fragments.

The arm angle (or carrying angle at the elbow joint), which is the angle between the long axes of the humerus and ulna (arm angles $<0^\circ$ imply medial deviation of the ulna) (51), was computed from a photograph of the rearticulated original specimens.

Postcranial proportions

The degree of elongation of the forearm, the arm, and the forelimb as a whole were assessed by means of allometric regressions of radius, humerus, and radius-plus-humerus length (in millimeters), respectively, relative to BM (in kilograms). Allometric equations (table S4) were derived based

on data taken from the literature (48, 52–54) or kindly provided by E. Sarmiento to S.M.S. Regressions for anthropoid primates were based on $n = 54$ sex-species means, corresponding to 31 species from 17 genera; hylobatids, orangutans, *Ateles*, and *Brachyteles* were excluded from the regressions because they are clear outliers. Allometric residuals were computed for the studied sample as well as for *Pliobates* and *Epipliopithecus*; humeral length in the former, given the lack of the humeral head, was estimated by taking into account the proportions of the humerus of *Epipliopithecus*.

To assess the size of the triquetrum relative to the hamate, the size of each bone was computed as the geometric mean of three measurements representing their maximum dimensions: maximum mediolateral breadth, dorsopalmar height, and proximodistal length for the triquetrum; and maximum proximodistal length, dorsopalmar height, and mediolateral breadth for the hamate. Comparative data were kindly provided by T. Kivell; her metrics and sample (including 28 anthropoid species from 16 genera) are described in the literature (55, 56).

Cranial capacity and encephalization

For preservational reasons, it was not possible to compute cranial capacity (CC, in cubic centimeters) from the virtual endocast. Therefore, CC was estimated using published allometric equations based on various external neurocranial measurements (57): maximum width of the braincase base (CW, in millimeters); vertical height of braincase (CH, in millimeters); chord of the midline suture through occipitals, parietals, and frontals (CL, in millimeters); modulus of the above-mentioned linear measurements (CO, in millimeters), computed as $CO = CW + CH + CL$; the product of the above-mentioned linear dimensions (PR, in cubic millimeters), computed as $PR = CW \times CH \times CL$; and foramen magnum area (FMA, in square centimeters), computed as $FMA = (\pi/4) \times FMW \times FML$, where FMW is foramen magnum width, and FML is foramen magnum length (both in millimeters). To assess encephalization, we relied on lower taxonomic-level metrics of relative brain size, which are significantly correlated with general domain cognitive abilities in primates (58, 59). Encephalization residuals (ER) were computed as $ER = \ln CC_{\text{observed}} - \ln CC_{\text{predicted}}$, by using the ordinary least-squares cercopithecoid allometric regression ($\ln CC = 0.4778 \ln BM + 3.457$), whereas encephalization constants (EC) were computed as $EC = CC / BM^{0.28}$ (58, 59). Sex-species mean data for extant species were taken from the literature (58). In addition to the estimates derived for *Pliobates* in this study, published data for various extinct catarrhines (female *Aegyptopithecus zeuxis*, male *Victoriapithecus macinnesi*, female *Proconsul nyanzae*, male *Oreopithecus bambolii*, and female *Hispanopithecus hungaricus*) were also included in the analyses (37, 58, 60–62).

Dental microwear

Paleodietary reconstruction was based on dental microwear analysis (63). The M1 of *Pliobates* was

selected, because it exhibits clearer and larger phase II crushing and grinding facets than the remaining molars. Occlusal surfaces were examined through an environmental scanning electron microscope at $\times 500$ magnification in secondary emissions mode and at 20 kV, following established procedures (63). An area of standardized size, corresponding to 0.02 mm² on the original facet (64), was analyzed using the custom software package Microware 4.02 (65). Three microscopic variables were measured: percentage of pits, breadth of striations, and breadth of pits. Striations and pits were categorized by following an arbitrarily set length-to-width ratio of 4:1 (63, 66–68). We compared our results with those previously derived for a sample of 11 extant anthropoids with well-known diets (63, 69, 70), partitioned into three distinct dietary categories (66), as well as with those reported for extinct catarrhines (64, 66–68, 71), including both pliopithecoids and hominoids. Microwear data were analyzed by means of canonical variates analysis, using SPSS Statistics 19 software.

Phylogenetic analysis

A cladistic analysis based on maximum parsimony was performed with PAUP* (Phylogenetic Analysis Using Parsimony) version 4.0 for Unix (72), with the search command “branch-and-bound,” based on a taxon-character data matrix of 319 characters and 20 taxa (tables S5 and S6). This matrix was coded anew by the authors, although it was partially based on character statements taken from the literature (8, 22, 73–76). All but 10 characters were treated as unordered, whereas inapplicable characters were treated as missing data. Clade robusticity was assessed by means of bootstrap analysis (10,000 replicates) and Bremer support indices. For the most-parsimonious cladograms, the following metrics were computed: consistency index (excluding uninformative characters), retention index, and rescaled consistency index. Character polarity was determined using the outgroup method, with the stem catarrhine *Aegyptopithecus* being used as such. Ingroup taxa include the stem catarrhine *Saadanius*, two cercopithecoids (the extant *Macaca* and the extinct *Victoriapithecus*), extant hylobatids, extant (*Pongo*, *Gorilla*, and *Pan*) and extinct (*Pierolapithecus* and *Hispanopithecus*) great apes, and a wide representation of small-bodied fossil catarrhines from Africa [two dendropithecids, including *Micropithecus* and *Dendropithecus*-plus-*Simiolus* (coded simultaneously)] and Eurasia (six pliopithecoids, including *Pliopithecus*, *Epipliopithecus*, *Diomyopithecus*, *Barberapithecus*, *Anapithecus*, and *Plesiopliopithecus*). The phylogenetic placement of *Saadanius*, *Micropithecus*, and most pliopithecoids (except *Epipliopithecus*) should be considered with caution, because they have a large proportion of missing data. For this reason, the analysis was also performed with these taxa removed.

Systematic paleontology

Order Primates Linnaeus, 1758. Suborder Haplorrhini Pocock, 1918. Infraorder Anthropoidea Mivart, 1864. Parvorder Catarrhini É. Geoffroy Saint-Hilaire, 1812. Superfamily Hominoidea Gray,

1821. Family Pliobatidae fam. nov. Type genus: *Pliobates* gen. nov., whose diagnosis is as for its type (and only) species, described below.

Pliobates cataloniae gen. et sp. nov.

Holotype: IPS58443, a partial skeleton with an associated skull (Fig. 1 and movie S1), housed at the ICP. It is composed of 70 bones and bone fragments (table S1) found in close spatial association, which, given the lack of repeated elements, are attributed to a single adult female individual (based on the small canine alveolus), with an estimated body mass of 4 to 5 kg (tables S7 and S8). It includes large portions of the cranium with postcanine maxillary teeth (Table 1), a mandibular fragment, a partial left forelimb (nearly complete humerus, radius, partial ulna, carpals, and bones of the manual rays), more fragmentary elements of the right forelimb, and bones from the hind limb.

Type locality: ACM/C8-A4 (els Hostalets de Pierola, Catalonia, Spain), in the ACM stratigraphic series (Vallès-Penedès Basin, northeast Iberian Peninsula).

Age, stratigraphic position, and distribution: Only known from the type locality, which has an estimated age of 11.6 Ma (middle/late Miocene boundary) and is thus somewhat younger than all other ACM hominoid- and pliopithecoid-bearing localities (9, 31, 33, 77, 78), the latter of which have been dated to 11.7 to 11.9 Ma [updated from (77, 78)].

Etymology: Genus name from the Latin *plio*- (itself from the Greek, meaning “greater in extent”) and from the Greek *bates* (meaning “the one that walks or haunts”). The name is a contraction of the genus names *Pliopithecus* (“more ape”) and *Hylobates* (“the one that walks in the woods or in the trees”), in allusion to the small body size and the mosaic of primitive (stem catarrhine-like) and derived (crown hominoid) features displayed by the new taxon. The species epithet is the genitive of the female substantive “Catalonia,” the Latin name of Catalunya (in which the type locality is situated).

Diagnosis

Small-bodied catarrhine primate (estimated female BM of 4 to 5 kg). Dental formula 2.1.2.3.

Female upper canines moderately compressed. Upper cheek teeth low-crowned and with subpyramidal, moderately peripheral, and inflated cusps. Upper premolars relatively broad and ovoid, P4 smaller than P3, both with heteromorphic cusps, a markedly convex lingual contour and a distinct lingual cingulum (more developed in the P4), a distinct transverse crest separating the restricted mesial fovea from the extensive trigon basin, and the postparacrista forming an abrupt angle with the distal marginal ridge. Upper molars only moderately broader than long, with markedly convex lingual profiles; buccal cusps quite peripheral and buccal cingula discontinuous; lingual cingula relatively well developed, shelf-like, and C-shaped, but not surrounding the hypocone (which is distinct and more peripheral than the protocone); mesial fovea restricted, with an obliquely directed preprotocrista, and trigon basin extensive, being separated by a continuous crista obliqua from the slightly smaller distal fovea, which displays no hypocone-metacone crest. M2 slightly larger than the M1, and M3 shorter and trapezoidal (due to the oblique buccal margin, with a centrally situated metacone and a rudimentary hypocone).

Face small but with a distinct snout, the anterior portion of the nasals being almost parallel to the palate. Maxillary sinus large and frontal sinus present but small. Nasal aperture narrow. Nasoalveolar clivus short, with an open palatine fenestra. Anteriorly slightly narrow palate with somewhat convergent upper tooth rows. Zygomatic root moderately high. Orbits subcircular, large, and frontated, with telescopic orbital rims located over the P4. Estimated cranial capacity (69 to 75 cm³) indicating a monkey-like degree of encephalization. External auditory meatus tubular but short and not completely ossified, with a V-shaped end and its anterior portion fused with the postglenoid process. Carotid foramen perforating the bulla posterodistally, and carotid canal horizontally and anteriorly oriented. Spinosum and postglenoid foramina absent. Jugular foramen large and ventrally visible.

Humerus without entepicondylar foramen and capitular tail, with a well-developed capitulum, and a narrow and deep zona conoidea. Radial head rounded and not very tilted, with a markedly

Table 1. Dental measurements. Standard dental measurements to assess dental size and proportions were taken to the nearest 0.1 mm in the holotype (IPS58443) of *Pliobates cataloniae* gen. et sp. nov. MD, mesiodistal length (in millimeters); BL, buccolingual width (in millimeters); BLI, breadth/length index (in percent), computed as BL/MD $\times$ 100. Dashes indicate lack of data due to incomplete preservation.

Catalog no.	Tooth	Side	MD	BL	BLI
IPS58443.1	P3	Right	>2.8	>4.5	—
IPS58443.1	P4	Right	3.2	5.3	165.6
IPS58443.1	M1	Right	5.0	5.9	118.0
IPS58443.1	M2	Right	5.3	6.5	122.6
IPS58443.1	M3	Right	4.6	6.4	139.1
IPS58443.2	M2	Left	>5.0	—	—
IPS58443.2	M3	Left	4.5	6.7	148.9

beveled surface for articulation with the humeral zona conoidea, the articular surface for the ulnar radial notch extending along a large portion of the radial head, and a laterally facing bicipital tuberosity. Distal radioulnar joint fully diarthrodial, with an expanded and two-faceted semilunar articulation on the ulnar head, and a partially developed ulnar fovea. Ulnar styloid process with reduced girth and not articulating with the short pisiform. Triquetrum small and with a reduced articular surface for the ulnar styloid process. Hamate relatively long proximodistally, with a steep triquetrum facet, a relatively large head and a distally projecting hamulus. Capitate with a relatively small and oblong head and a divided facet for the second metacarpal on its radial side.

Differential diagnosis

The new taxon differs from pliopithecoids and dendropithecids in its lack of a humeral caputular tail, its hominoid-like proximal radial morphology, its expanded ulnar head with a two-faceted semilunar articulation, and its partially developed ulnar fovea. It further differs from these taxa and proconsulids in its more hominoid-like carpal morphology (including the lack of a pisiform facet for the styloid process, a capitate facet for the second metacarpal divided by a deep ligamentary notch, and a distally projecting hamulus in the hamate), and particularly from pliopithecoids in its overall larger muzzle, more horizontal nasals anteriorly, some details of the upper molars, and (at least compared with *Epipliothecus*) the lack of an entepicondylar foramen in the humerus. It also differs from all of the above-mentioned taxa in its fused ectotympanic and postglenoid process, and from these taxa and hominids in its horizontal and anteriorly oriented carotid canal. Last, it differs from crown hominoids (hylobatids and hominids) in its incompletely ossified ectotympanic and in its more primitive dentition and forelimb morphology (particularly in the humero-ulnar articulation).

Description, comparisons, and paleobiology

Dental morphology and diet

Although the lack of lower dentition precludes comparisons with some taxa, the upper cheek teeth of *Pliobates* (Fig. 1D and Table 1) generally resemble those of other small-bodied Miocene catarrhines in both occlusal morphology and proportions (figs. S1 and S2). In contrast, they display a more primitive morphology than those of extant hominoids, including the similarly sized gibbons. Hylobatids possess more elongated cheek teeth with more peripheralized cusps, less developed cingula, and a much more extensive central fovea. Compared with Miocene small-bodied catarrhines from Eurasia and Africa (fig. S1), the upper molars of *Pliobates* more closely resemble those of the dendropithecoid *Microplithecus* (21, 34, 35, 79) in several features, such as the markedly convex lingual profiles and moderately developed buccal cingula (albeit to a lesser extent than in *Microplithecus*), the C-shaped lingual cingulum that is mostly restricted to the protocone (not surround-

ing the hypocone), the well-developed and lingually situated hypocone, and the relatively narrow M1 and M2. Nevertheless, *Pliobates* differs in several features from *Microplithecus*, which has more restricted buccal cingula, a hypocone-metacene crest, and a relatively longer and less trapezoidal M3. The dentition of *Pliobates* more clearly differs from *Epipliothecus* and other pliopithecoids (fig. S1), including from *Barberapithecus* [also recorded at the Vallès-Penedès Basin (36)] and *Pliopithecus* [previously recorded at ACM (33)] in several features, such as the more convex lingual profile, the more peripheral buccal cusps, the less developed cingula of the molars, the narrower M1 and M2, and the M3 occlusal morphology and proportions (fig. S2).

With regard to microwear features, the M1 displays a pitting percentage of 30.0%, a pit breadth of 5.67 μm , and a striation breadth of 1.98 μm . Based on pitting incidence (Fig. 2, A and B), which is the most useful metric for distinguishing among dietary categories (70), *Pliobates* closely

resembles extant frugivores (*Pan troglodytes*) and eclectic feeders (*Papio cynocephalus*) that largely rely on ripe fruit. In contrast, the pitting incidence of *Pliobates* is higher than in extant folivores and much lower than in extant hard-object feeders (including orangutans). Compared with other extinct catarrhines from Western Europe, the pitting percentage of *Pliobates* is somewhat lower than in most pliopithecoids and hominoids, for which some degree of sclerocarpus has been inferred (66, 68). This low pitting incidence is consistent with the pit- and striation-breadth measurements (which show no sign of extreme folivory or specialized hard-object feeding) and most closely approaches that of the fossil hominoids *Anoiapithecus brevirostris* and *Hispanopithecus laietanus*, previously interpreted as soft frugivores (68). These results are confirmed by a multivariate analysis that simultaneously examined the three microwear variables (Fig. 2C and tables S9 to S11), in which *Pliobates* falls closer to the extant frugivorous-mixed-feeder centroid for the first

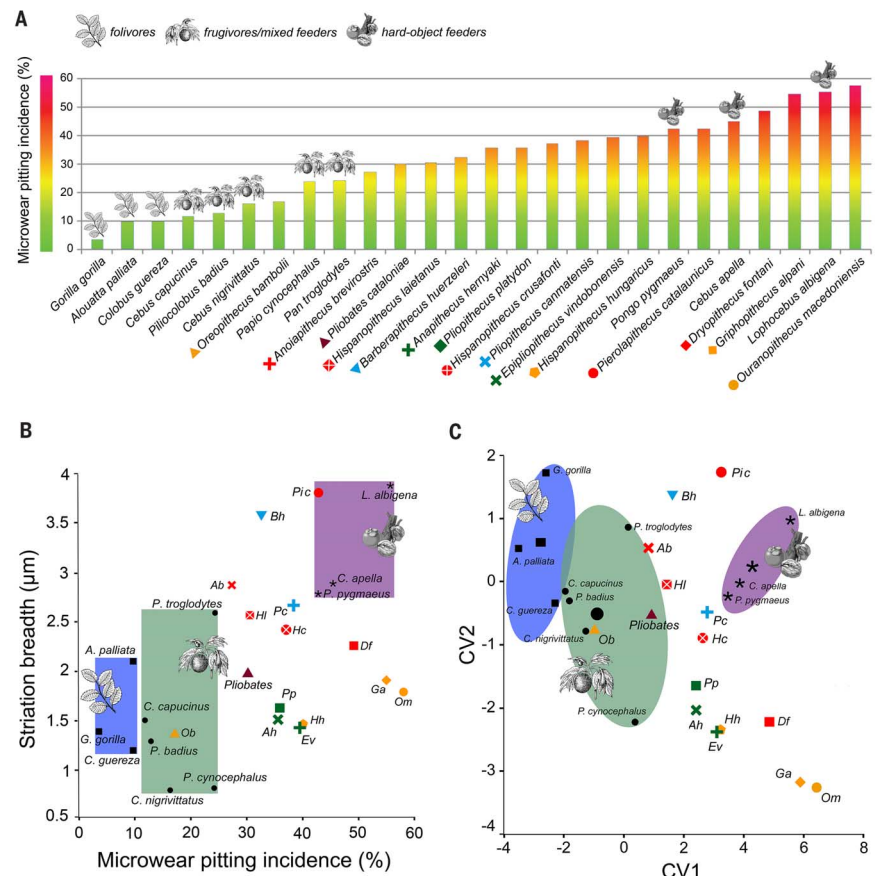


Fig. 2. Results of the dental microwear analyses. (A) Pitting incidence (%) of *Pliobates*, the extant comparative sample, and pliopithecoids and extinct hominoids from Europe and Turkey. (B) Bivariate plot of striation breadth versus pitting incidence. (C) Bivariate plot of the first two canonical axes delivered by the canonical variates analysis, based on three distinct, broad dietary groups: folivores, mixed feeders and frugivores, and hard-object feeders. Colored polygons in (B) and ellipses in (C) illustrate the variability of extant dietary categories. Small black symbols denote the comparative sample of extant anthropoids, whereas large black symbols represent the centroids of each dietary category. Different symbols are employed to distinguish the various extinct species; results for Iberian hominoids and pliopithecoids are shown in red and blue, respectively, whereas those from other localities are shown in yellow and green, respectively.

and second canonical axes and is classified as a frugivore. Dental microwear analyses therefore indicate a mainly frugivorous diet for *Pliobates*, compatible with a high consumption of ripe fruit and a low sclerocarpic component.

Body mass

Dental BM estimates for the female holotype of *Pliobates* (table S7) range from 2.9 to 4.8 kg, with an average BM estimate of 3.9 kg and an uncertainty degree (based on the combined 95% confidence intervals for each dental locus) of 2.5 to 5.7 kg. Postcranial BM estimates (table S8) are on average 4.8 kg (range: 4.0 to 5.6 kg), based on catarrhine regressions, and 4.3 kg (range: 2.6 to 6.3 kg), based on hominoid regressions (estimates for each postcranial estimator and their confidence intervals are given in table S8). Given that the size of *Pliobates* is in the lower range for extant hominoids, the catarrhine regressions probably yield more accurate estimates, although the hominoid-based estimates are closer to the dental ones. Overall, the body mass of the holotype of *Pliobates cataloniae* can be estimated at ~4 to 5 kg (much lower than that estimated for *Epipliopithecus vindobonensis*, ~11 to 12 kg; table S8).

Cranial morphology and encephalization

A 3D virtual reconstruction of the cranium, based on the preserved specimens, is shown in fig. S3, whereas the final reconstruction (including mirrored portions) is shown in Fig. 3 and movie S1. Based on this reconstruction, the cranium of *Pliobates* differs from the primitive catarrhine condition (22, 37, 60) by being short, wide, and high. However, the tubular ectotympanic is short and incompletely ossified—i.e., less developed than in *Saadanius* and extant crown catarrhines (20–22). The maxillary sinus is extensive, as in stem catarrhines and hominoids (22, 80), and there is also a small frontal sinus, as in stem hominoids but unlike in stem catarrhines, cercopithecoids, hylobatids, and pongines (22, 37, 80). The face is short and displays anteriorly situated orbits, as in hylobatids, colobines, and some extinct small-bodied catarrhines such as *Epipliopithecus*, *Micropithecus*, and *Lomorupithecus* (19, 21, 38, 76, 79). However, *Pliobates* differs from these taxa (and more closely resembles hylobatids) by displaying a more well-defined muzzle (especially compared with *Epipliopithecus*) with long and more horizontal nasals, a higher zygomatic root (moderately high as in hylobatids, but less so than in hominids), an interorbital pillar nearly orthogonal to the frontal squama (as in hylobatids and chimpanzees), a high degree of orbital convergence and frontation (as in all extant hominoids), and thin and anteriorly projecting (telescopic) orbital rims [to a greater extent than in *Epipliopithecus* (38), and thus most closely resembling hylobatids and, as far as it can be ascertained with incomplete preservation, *Micropithecus* (79)]. *Pliobates* also displays derived hominoid features in the basicranium (Fig. 4A), including the absence of a postglenoid foramen with a large and ventrally visible jugular foramen (as in all extant hominoids), the foramen ovale situated anteriorly and laterally to the Eusta-

chian aperture (as in hylobatids and African apes), the fusion between the auditory meatus and the postglenoid process (as in hylobatids

and African apes), and the horizontal and anteriorly directed carotid canal in the petrosal bone (as in hylobatids).

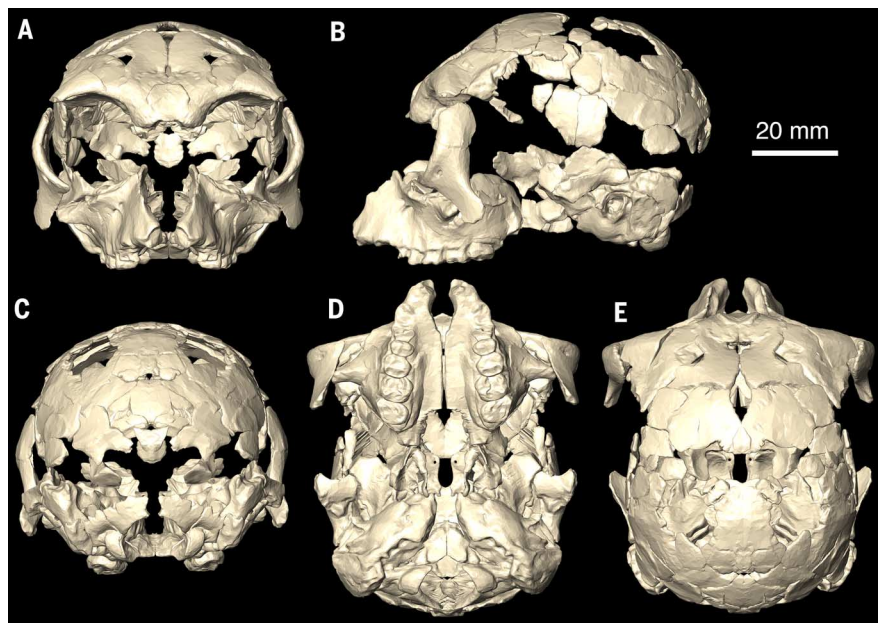


Fig. 3. Cranial reconstruction. Virtual reconstruction of the holotype (IPS58443) cranium of *Pliobates cataloniae* gen. et sp. nov., including mirrored fragments, in frontal (A), lateral (B), posterior (C), basal (D), and superior (E) views. Further details are given in fig. S3 and the methods in the text.

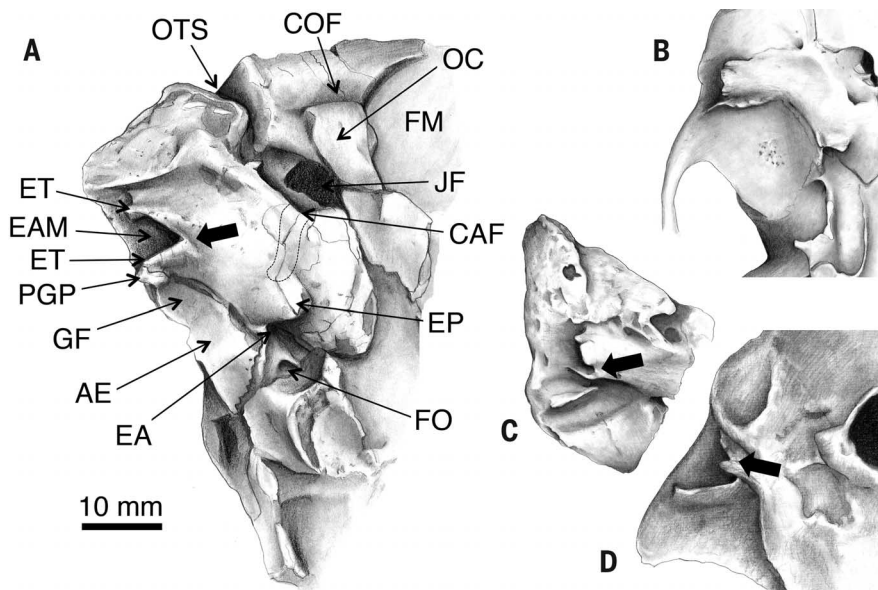


Fig. 4. Basicranial morphology. (A) Drawing of the left basicranium of the holotype (IPS58443) of *Pliobates cataloniae* gen. et sp. nov., as preserved in ventral view. The jugular foramen appears artifactually larger because of the displacement of the temporal and occipital portions along the occipitotemporal suture (corrected in the reconstruction in Fig. 3). The course of the carotid canal is shown with a dashed line, based on CT images. AE, articular eminence; CAF, carotid foramen; COF, condylar fossa; EA, Eustachian aperture; EAM, external auditory meatus; EP, Eustachian process; ET, ectotympanic; FM, foramen magnum; FO, foramen ovale; GF, glenoid fossa; JF, jugular foramen; OC, occipital condyle; OTS, occipitotemporal suture; PGP, postglenoid process. (B to D) Drawings of comparable views (not to scale) in *Hylobates* sp. (B), *Proconsul heseloni* KNM RU 2036 [(C), reversed], and *Victoriapithecus macinnesi* KNM MB 29100a (D) (KNM, Kenyan National Museums; RU, Rusinga; MB, Maboko). Arrows denote the V-shaped, incompletely ossified ventral terminal tip of the tubular ectotympanic in the extinct taxa. [Artwork by M. Palmero]

Braincase measurements yield an average cranial capacity estimate of 69.0 cm³ (range: 41.4 to 110.7 cm³; table S12), which is close to the estimates of 60.1 and 65.3 cm³ delivered by the two most reliable estimators (57) and only slightly lower than the estimate of 75.1 cm³ obtained from foramen magnum area (table S12). According to our estimates of body mass (4.5 kg) and cranial capacity [72 cm³ (average of cranial and foramen magnum estimates)], *Pliobates* would display a monkey-like degree of encephalization extensively overlapping with extant cercopithecoids (fig. S4 and table S13), being much more encephalized than the stem catarrhine *Aegyptopithecus*, slightly more so than the stem cercopithecoid *Victoriapithecus*, and only slightly less so than hylobatids and the extant hominoids *Proconsul* and *Oreopithecus*. All these taxa, like cercopithecoids, are less encephalized than the extinct hominoid *Hispanopithecus* (*Rudapithecus*) and the extant great apes. Although humans are outliers in brain size–body size allometric regressions, great apes further display an allometric grade shift compared with hylobatids (and *Pliobates*), which are only slightly more encephalized on average than cercopithecoids (58).

Postcranial morphology and locomotion

The humerus (Fig. 5) resembles that of extant crown catarrhines, proconsulids, and dendropithecids by lacking (unlike *Epipliopithecus*) an entepicondylar foramen (17, 20, 21, 38, 79, 81, 82). *Pliobates* more closely resembles extant hominoids in the laterally facing bicipital tuberosity in the radius (81, 83) (Fig. 5), as well as in the configuration of the humero-radial articulation (81, 82, 84) (Fig. 6), including: in the humerus, the lack of capitular tail [present in *Epipliopithecus*, dendropithecids, and cercopithecoids (84)] and the moderately globulous (although not posterolaterally expanded) capitulum with a well-developed zona conoidea [lacking in *Epipliopithecus* and dendropithecids (81–84)]; and, in the radius, the only slightly tilted and almost circular radial head with a small and flat area, a reduced lateral lip, and a beveled surface for the humeral zona conoidea. *Pliobates* also has a hominoid-like diarthrodial distal radio-ulnar joint (85–87), with a two-faceted expanded semilunar articulation in the ulnar head (Fig. 6). In this regard, *Pliobates* departs from *Epipliopithecus*, dendropithecids, and cercopithecoids (17, 38, 82) and more closely resembles *Proconsul* (88), although the ulnar head is less extensive than in extant hominoids. In contrast with these derived features, the humeral shaft and humero-ulnar joint are plesiomorphic: The former (Fig. 5) is anteriorly straight and somewhat proximally retroflexed; the latter (Fig. 6) lacks the stabilizing features of extant hominoids (83, 89), as shown by the narrow ulnar trochlear notch without a median keel (in agreement with the absence of spooling and the poorly defined trochlear lateral keel in the humerus of *Pliobates*).

Humeral torsion in *Pliobates* is estimated at 101°, irrespective of the method employed (based on the posterior buttress for the humeral head or the bisector of the bicipital groove), with a confidence interval spanning 95.7° to 106.3° [based

on the prediction error (5.23%) for the bicipital groove method (50)]. This degree of torsion is moderate, higher than that estimated for *Proconsul heseloni* (92°), but comparable to estimates for *Dryopithecus fontani* (102°) and *Dendropithecus macinnesi* (103.5°), and only slightly below the value estimated for *Epipliopithecus vindobonensis* (109°). The humeral torsion of *Pliobates* is thus most comparable to that of non-atelid platyrrhines and lower than that of *Ateles* and extant hominoids, especially African great apes and humans [although the high degree of humeral torsion of extant hominoids is related to increased mobility at the glenohumeral joint, the higher values of great apes and humans appear related to knuckle-walking and enhanced manipulation, respectively, rather than suspensory behaviors (50)]. In contrast to the moderate humeral torsion, the forelimb of *Pliobates* appears somewhat elongated relative to its body size (fig. S5). Allometric computations of relative forelimb length in fossils (residuals are given in table S14) must be considered with caution, because they are dependent on the accuracy of body-size estimates. However, the forelimb of *Pliobates* (based on our BM estimate of 4.5 kg) appears more elongated than that of *Epipliopithecus* (based on our estimate of 11.5 kg). The latter taxon, contrary to previous assertions (81, 83), has the generalized proportions of quadrupedal monkeys. *Pliobates*, in con-

trast, has a forelimb elongation similar to that of female orangutans and *Brachyteles*, although it is less extreme than in *Ateles* and especially than in hylobatids. The same pattern holds when the humerus and radius are analyzed separately, although in *Pliobates*, relative length is somewhat higher for the radius than for the humerus. *Pliobates* further displays a high arm angle (8°), which is considerably greater than the average in most anthropoids, except *Hylobates* (9.8°), *Pongo* (6.3°), and *Ateles* (6.5°) (51).

The ulnocarpal articulation of *Pliobates* is completely different from that of *Epipliopithecus* and dendropithecids (17, 38, 90), including a partially developed ulnar fovea (Fig. 6), which, in extant hominoids, is the attachment area of the triangular disc ligament and the intra-articular meniscus (85–87). The ulnar styloid process is relatively long and slender, with no discernible articular surfaces for the pisiform or triquetrum. This agrees with the lack of an articular facet for the styloid process on the pisiform, like extant hominoids but unlike monkeys and *Proconsul* (91). However, in contrast to *Pierolapithecus* (92), the triquetrum of *Pliobates* shows a proximal articular facet, which is more developed than that present in hylobatids and sometimes *Pan* (51, 87) but less developed than in monkeys. This suggests that ulnotriquetral contact might have been reduced by some kind of intra-articular

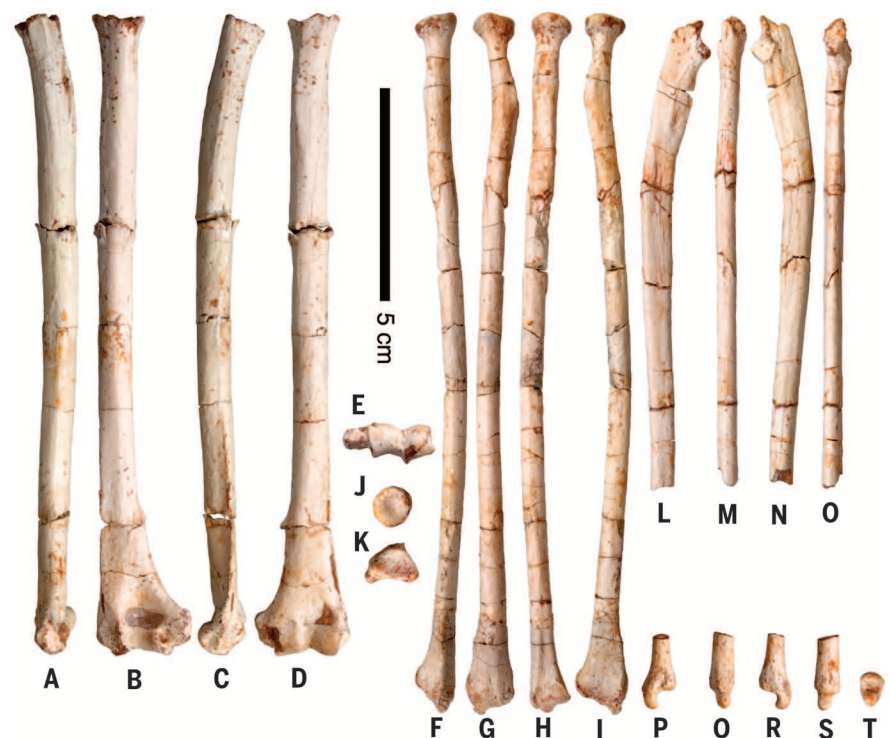


Fig. 5. Forelimb long bones. Shown are the humerus, radius, and ulna of the holotype (IPS58443) of *Pliobates cataloniae* gen. et sp. nov. (A to E) Partial left humerus in medial (A), posterior (B), lateral (C), anterior (D), and distal (E) views. (F to K) Left radius in medial (F), posterior (G), lateral (H), anterior (I), proximal (J), and distal (K) views. (L to O) Proximal half of the left ulna in medial (L), posterior (M), lateral (N), and anterior (O) views. (P to T) Distal fragment of the left ulna in medial (P), posterior (Q), lateral (R), anterior (S), and distal (T) views.

tissue, similarly to some *Ateles* species (93). Moreover, as in apes, the triquetrum of *Pliobates* is small relative to hamate size (fig. S6), indicating a reduced loading on the ulnar side of the wrist. However, as in monkeys and *Proconsul*, the triquetrum of *Pliobates* differs from that of extant apes and *Pierolapithecus* (92) by possessing a proximally protruding beak-like process (Fig. 7). The hamate of *Pliobates* is “Miocene ape-like” (91, 92), although it more closely resembles that of hylobatids by possessing a dorsopalmarly narrow and proximodistally long triquetral articular surface that is proximally globular, as well as a distally projecting hamulus (Fig. 7). *Pliobates* further resembles hylobatids and *Ateles* by having an oblong and mediolaterally narrow capitate head that, like the facet for the hamate, is proximodistally aligned. This morphology contrasts with the more globulous, wider, and ulnarly inclined capitate head of other catarrhines, including *Proconsul* (88, 91) and *Pierolapithecus* (92); in *Pliobates*, though, it is not radially inclined, as it is in hylobatids. Moreover, the capitate facet for the second metacarpal is divided by a deep ligamentary notch (Fig. 7), as in extant hominoids and *Pierolapithecus* (92) but not in other catarrhines (including *Proconsul*), in which the facet for the second metacarpal is dorsopalmarly continuous and occupies the whole lateral aspect of the capitate (Fig. 7) (91). *Pliobates* has a complex articulation between the third metacarpal and capitate, as in extant apes but not in *Proconsul* (91); however, as in *Pierolapithecus* (92), the capitate of *Pliobates* lacks a hook-like process.

Regarding positional behaviors, although the primitive morphology of the proximal humerus of *Pliobates* is suggestive of generalized above-branch quadrupedalism (94), its overall postcranial body plan is more compatible with a locomotor repertoire that includes a large amount of cautious and eclectic climbing (87, 95). This inference is supported by the emphasis on pronation and supination capabilities, the reduced compressive forces transferred across the ulnar side of the wrist, and the important ulnar deviation and rotatory capabilities. It agrees with previous hypotheses on the original locomotor adaptations of hominoids (95, 96) and with recent interpretations of *Proconsul* that similarly depict this taxon as an arboreal quadruped with adaptations for cautious climbing and clambering (12, 88), including an incipient distal radioulnar diarthrosis that (unlike in *Pliobates*) is still associated to a nonretreated ulnar styloid process (88). The reduced ulnocarpal articulation of *Pliobates* thus more closely foreshadows the condition of extant hominoids, although to a lesser extent than in the stem great ape *Pierolapithecus* (92), indicating a decreased emphasis on forearm use under weight-bearing conditions relative to *Proconsul* (88). Several characteristics of *Pliobates* (particularly the elongated forearm, the high arm angle, and the laterally facing bicipital tuberosity) further suggest some degree of below-branch forelimb-dominated suspensory behaviors (51). However, the lack of hominoid-like elbow-stabilizing features in the humeroulnar joint (83, 89), the generalized

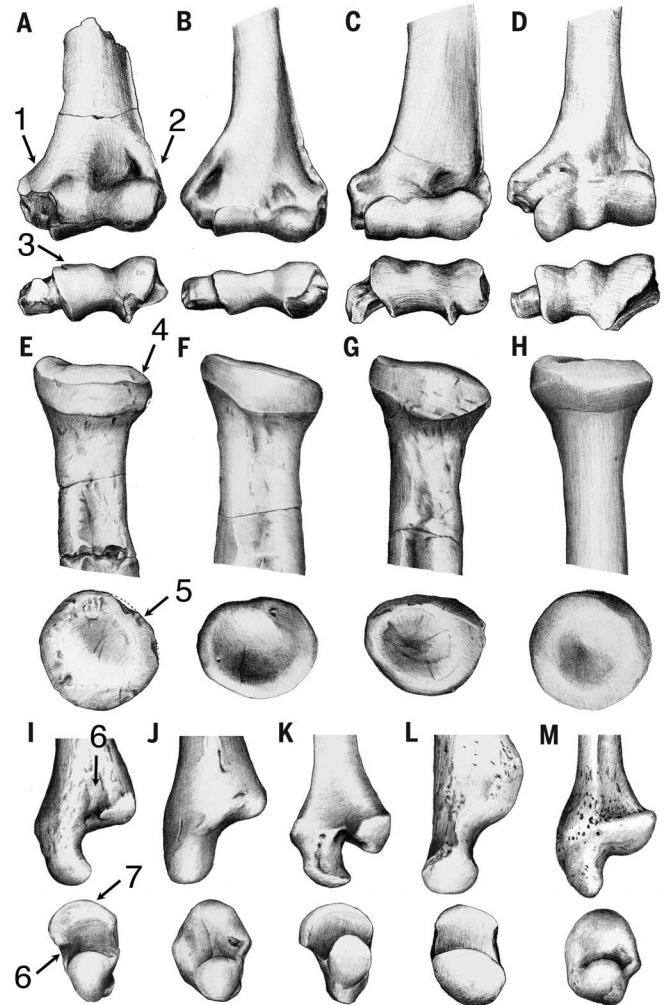
metacarpophalangeal proportions, and the lack of marked phalangeal curvature suggest that *Pliobates* was not specifically adapted to perform the acrobatic suspensory behaviors (ricochetal brachiation) displayed by extant gibbons.

Phylogeny and evolutionary implications

Our cladistic analysis, based on both craniodental and postcranial characters, recovers a single most-parsimonious tree (Fig. 8 and table S15), indicating that *Pliobates* is more closely related to crown hominoids than other Miocene small-bodied catarrhines and *Proconsul* are. With moderate support (bootstrap 71%, Bremer index 3), our results contradict the view of some authors that all of these taxa are stem catarrhines (preceding the divergence between hominoids and Old World monkeys) (17, 21) and concur instead with some previous cladistic analyses indicating a hominoid status for both *Proconsul* (2, 8, 22) and, at least, dendropithecids (8, 22). Our analysis is inconsistent with the current consensus that

Epipliopithecus is a pliopithecoid (20, 66, 76, 78); however, the internal phylogeny of pliopithecoids and dendropithecids is not settled by our results (it is recovered by the most-parsimonious tree but not by the bootstrap 50%-majority-rule consensus). Similarly, the phylogenetic relationships between the analyzed dryopithecines (*Hispanopithecus* and *Pierolapithecus*) are not well resolved (the closer link between *Pierolapithecus* and crown hominoids has a Bremer index of 1 and bootstrap support of 56%). In contrast, our analysis recovers, with very high support (bootstrap 93 to 100%, Bremer indices 7 to 11), both the molecular phylogeny of extant hominoids (1, 6) and the stem hominid status of *Pierolapithecus* and *Hispanopithecus* (9, 92). The position of *Pliobates* as a stem hominoid more derived than *Proconsul* is relatively well supported (bootstrap 78%, Bremer index 2). When the analysis is repeated excluding all fossil taxa with large amounts of missing data (fig. S7), the position of *Pliobates* as a stem hominoid more derived than *Proconsul* is much better

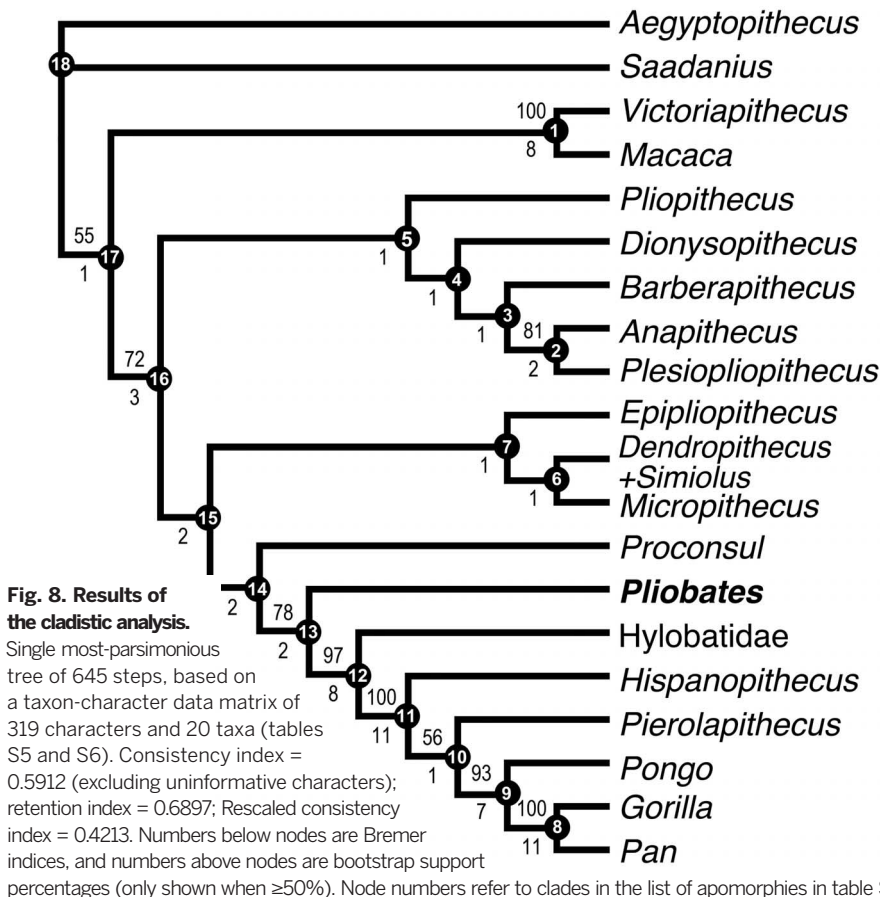
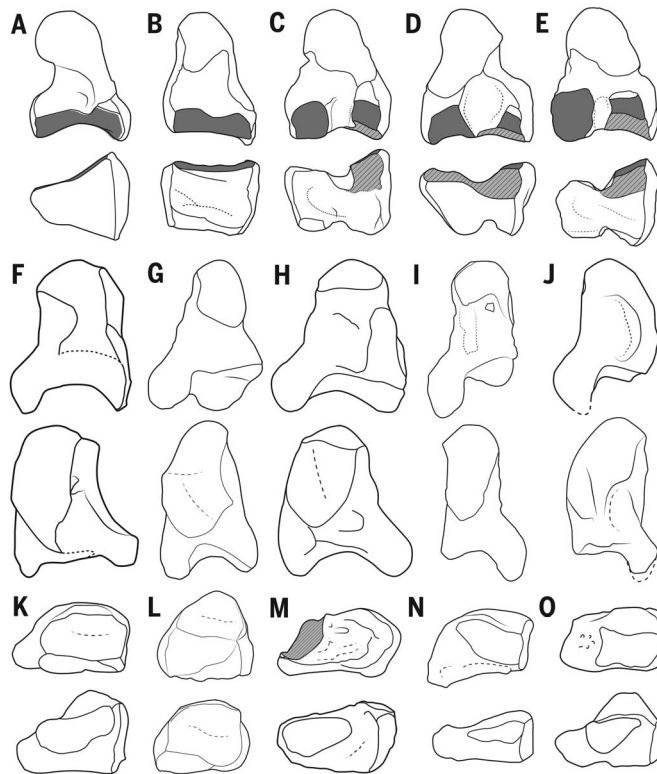
Fig. 6. Elbow and wrist morphology. The most diagnostic features of the elbow and wrist joints of *Pliobates cataloniae* gen. et sp. nov. (IPS58443), denoted by arrows in drawings of the distal humerus, proximal radius, and distal ulna, are shown with those of selected extant and extinct anthropoids for comparison. (A to D) Anterior (top) and distal (bottom) views of the distal humerus in *P. cataloniae* (A), *Epipliopithecus vindobonensis* Individual I [(B), reversed], *Dendropithecus?* sp. KNM MO 17022A (C) (MO, Moruorot), and *Hylobates moloch* (D). (E to H) Views perpendicular to the radial tuberosity (top) and proximal view (bottom) of the proximal radius in *P. cataloniae* (E), *E. vindobonensis* Individual I (F), *Simiolus enjessi* KNM MO 63 [(G), reversed], and *H. moloch* (H). (I to M) Medial (top) and distal (bottom) views of the distal ulna in *P. cataloniae* (I), *E. vindobonensis* Individual I (J), *H. moloch* (K), *Ateles paniscus* (L), and *Cercopithecus aethiops* (M). 1, absence of entepicondylar foramen; 2, absence of capitular tail; 3, lack of spool-shaped trochlea; 4, well-developed beveled surface for the zona conoidea; 5, small and flat area in the radial head; 6, ulnar fovea; 7, two-faceted, expanded semilunar articular surface in the ulnar head. Specimens are shown as if from the left side and are not to scale. [Artwork by M. Palmero]



Ateles paniscus (L), and *Cercopithecus aethiops* (M). 1, absence of entepicondylar foramen; 2, absence of capitular tail; 3, lack of spool-shaped trochlea; 4, well-developed beveled surface for the zona conoidea; 5, small and flat area in the radial head; 6, ulnar fovea; 7, two-faceted, expanded semilunar articular surface in the ulnar head. Specimens are shown as if from the left side and are not to scale. [Artwork by M. Palmero]

Fig. 7. Carpal bones.

Line drawings of carpal bones in *Pliobates cataloniae* gen. et sp. nov. (IPSS58443) are shown with those of selected anthropoid genera for comparison. (A to E) Left capitate, in radial (top) and proximal (bottom) views, of *Cercopithecus aethiops* (A), *Ateles paniscus* (B), *Pierolapithecus catalaunicus* (C), *Hylobates lar* (D), and *P. cataloniae* (E); gray shading denotes articular areas for the second metacarpals, and cross-hatching denotes those for the third metacarpal. (F to J) Left hamate, in radial (top) and ulnar (bottom) views, of *C. aethiops* (F), *A. paniscus* (G), *Pi. catalaunicus* (H), *H. lar* (I), and *P. cataloniae* (J). (K to O) Left triquetrum, in proximo-medial (top) and distal (bottom) views, of *C. aethiops* (K), *A. paniscus* (L), *Pi. catalaunicus* (M), *H. lar* (N), and *P. cataloniae* (O). Drawings are not to scale.

**Fig. 8. Results of the cladistic analysis.**

Single most-parsimonious tree of 645 steps, based on a taxon-character data matrix of 319 characters and 20 taxa (tables S5 and S6). Consistency index = 0.5912 (excluding uninformative characters); retention index = 0.6897; Rescaled consistency index = 0.4213. Numbers below nodes are Bremer indices, and numbers above nodes are bootstrap support percentages (only shown when $\geq 50\%$). Node numbers refer to clades in the list of apomorphies in table S15.

supported (bootstrap 100%, Bremer index 20); it is even more robust than the monophyly of crown hominoids (bootstrap 98%, Bremer index 11) and than the great-ape status of *Pierolapithecus* and *Hispanopithecus* (bootstrap 100%, Bremer index 13).

Given that our analyses support *Pliobates* as a stem hominoid more derived than *Proconsul*, the mosaic of primitive and derived features displayed by the former taxon is of utmost relevance for interpreting the evolution of several key features among catarrhine primates. Many authors agree that homoplasy has played an important role in hominoid postcranial evolution (5, 9, 12, 92), but *Pliobates* shows a mosaic of primitive and derived features both in the cranium and the postcranium. For the cranium, this is best illustrated by the short tubular (but incompletely ossified) external auditory meatus with a V-shaped end, which would imply the independent acquisition of a more completely ossified ectotympanic in cercopithecoids and hominoids. This has previously been posited by some authors (20), and it is suggested to some extent by the stem catarrhine *Saadanius* (22), the stem Old World monkey *Victoriapithecus* (60), and the stem ape *Proconsul* (97); in these taxa, the ectotympanic, albeit slightly more developed, is still short, does not laterally exceed the post-glenoid process, and lacks a completely closed terminal ventral tip (Fig. 4). In contrast, several other cranial features of *Pliobates* are derived toward the crown-hominoid condition, generally more closely resembling hylobatids than hominids. Some of the similarities with gibbons (e.g., short face with a distinct muzzle and anteriorly situated telescopic orbits) may be size-related to a large extent (98), but others (horizontal and anteriorly directed carotid canal) are otherwise only known in hylobatids. Coupled with the fact that *Pliobates* chronologically fits within the long ghost lineage of hylobatids, from their divergence from hominids at ~17 Ma (1) until their putative oldest record (*Yuanmoupithecus*) at 8 to 7 Ma (10, 39), these similarities raise the possibility that *Pliobates* might be a stem hylobatid. This hypothesis is not favored by our total (craniodental plus postcranial) evidence-based cladistic analyses, which support instead a stem hominoid status for *Pliobates*, mostly because of the lack of various crown-hominoid postcranial synapomorphies. However, its hylobatid cranial features and small body size suggest that, at least in some respects, the last common ancestor of crown hominoids might have been more gibbon-like (or less great ape-like) than generally assumed (6, 16).

Furthermore, *Pliobates* supports the view that some small-bodied catarrhines played a more important role in the emergence of crown hominoids than has generally been assumed over the past decades. The postcranial evidence provided by Miocene great apes such as *Pierolapithecus* and *Sivapithecus* (9, 92, 99) indicates that the last common ancestor of crown hominoids must have been postcranially more primitive than it would be inferred to be exclusively on the basis of extant forms. This supports some degree of parallel

evolution in the postcranium between extant hylobatids and hominids (5), although not to such a great extent as if *Pliobates* was interpreted as a crown hominoid. As a stem ape, *Pliobates* cannot resolve whether many of the postcranial derived features shared by extant hylobatids and hominids are homologous (6) or homoplastic (5), although *Pliobates* does suggest that a suite of features in the humeroradial and wrist joints might be homologous. In these anatomical regions, *Pliobates* displays much more extensive postcranial synapomorphies of crown hominoids than those convergently displayed by atelids, including a diarthrodial radioulnar joint, an expanded ulnar head, an incipient ulnar fovea, a long and thin styloid process with reduced contact with the relatively small triquetrum, and a distally projecting hamulus on the hamate. *Pliobates* also displays incipient suspensory adaptations, although, based on currently available evidence, it is not possible to conclusively ascertain whether these were inherited by the last common ancestor of crown hominoids (and later secondarily lost in some fossil great apes such as *Sivapithecus* and *Pierolapithecus*) or whether they merely represent an independent acquisition of *Pliobates*'s.

Conclusions

Pliobates provides the first evidence of crown-hominoid postcranial synapomorphies in a Miocene small-bodied catarrhine, thus demonstrating a greater diversity in postcranial morphology and positional behaviors than previously recognized among this paraphyletic assemblage of taxa. Three decades ago, the degree of parallel evolution required to evolve hylobatids from small-bodied catarrhines such as dendropithecids, albeit conceivable, was considered unlikely in light of the available evidence (18), because of the numerous parallelisms that would be required in crown-hominoid (and even crown-catarrhine) features between hylobatids and hominids. Although the evidence provided by *Pliobates* reduces this morphological gap, this taxon still falls short of being supported as a hylobatid by our cladistic analyses, which strongly favor instead the monophyly of extant hominoids with all fossil small-bodied catarrhines excluded. However, unlike dendropithecids, which are currently interpreted as stem catarrhines (21) or hominoids more basal than *Proconsul* (22), *Pliobates* is unambiguously interpreted as more closely related to crown hominoids. Given its chronology and geographic location, as well as the retention of plesiomorphic dental and some postcranial features that resemble those of small-bodied catarrhines such as dendropithecids, *Pliobates* is likely to be a late-surviving offshoot of a small African stem hominoid more closely related to crown hominoids than *Proconsul* is. This has important implications for reconstructing the ancestral morphotype from which extant hominoids evolved: It suggests that some small-bodied catarrhines could have played a much more remarkable role in ape evolution than previously thought, and that the last common ancestor of crown hominoids was not necessarily great ape-like.

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SUPPLEMENTARY MATERIALS

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Figs. S1 to S7
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Movie S1

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RESEARCH ARTICLE SUMMARY

STRUCTURAL BIOLOGY

Structure of *Tetrahymena* telomerase reveals previously unknown subunits, functions, and interactions

Jiansen Jiang,* Henry Chan,* Darian D. Cash, Edward J. Miracco, Rachel R. Ogorzalek Loo, Heather E. Upton, Duilio Cascio, Reid O'Brien Johnson, Kathleen Collins, Joseph A. Loo, Z. Hong Zhou, Juli Feigon†

INTRODUCTION: Telomerase is a ribonucleoprotein complex that extends the telomere DNA at the 3' ends of linear chromosomes, thereby counteracting the loss of DNA from replication and nucleolytic processing. Although telomerase is largely inactive in somatic cells, it is active in stem cells and highly active in most cancer cell lines, where its activity is necessary for the cells' immortal phenotype. Thus, telomerase is an important regulator of aging, tumorigenesis, and stem cell renewal. Telomerase uses a template contained within the integral telomerase RNA (TER) and a telomerase reverse transcriptase (TERT) to synthesize multiple copies of the G-strand telomere repeat,

which is TTGGGG in *Tetrahymena*. Telomerase can synthesize telomere repeat DNA in vitro with only TERT and TER, but physiological function requires a variety of other proteins. Telomerase recruitment to telomeres is regulated by the cell cycle, where its activity requires interplay between telomere end-protection and telomerase proteins. Telomere end-maintenance also requires coordinated recruitment of telomerase and DNA polymerase α for synthesis of G and C strands, respectively.

RATIONALE: A detailed mechanistic description of telomerase and its interaction at telomeres has been hampered by a lack of structural

models, due to difficulties in obtaining samples of sufficient quantity and quality, as well as subunit complexity and flexibility and low sequence identity among subunits from different organisms. Unlike yeast and mammalian telomerase, *Tetrahymena* telomerase is constitutively assembled, making it possible to purify from cells and study all holoenzyme components in a stable complex. In addition to TERT and TER, the *Tetrahymena* telomerase holoenzyme contains the known proteins

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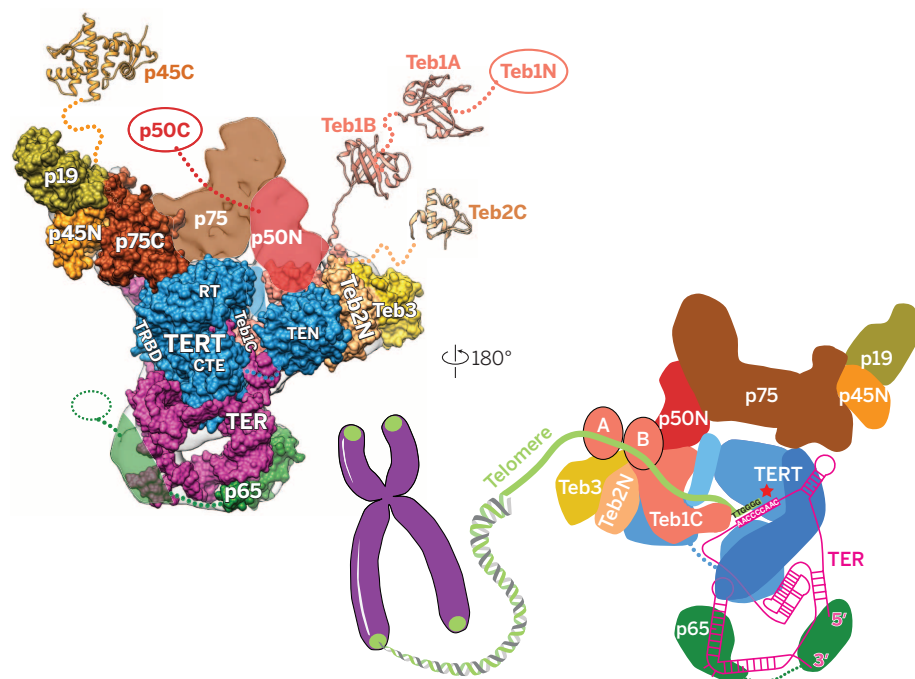
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p65, p75, p45, p19, p50, and Teb1, a telomeric G-strand binding protein that is paralogous to the large subunit of heterotrimeric replication protein

A (RPA). TER contains a template/pseudoknot domain enclosing a template with sequence complementarity to ~1.5 telomere repeats and a separate activating domain. Though TER is essential for activity, the physical arrangement of TER on TERT has remained largely unknown, as have details of protein subunit interactions.

RESULTS: We report cryo-electron microscopy (cryo-EM) structures at ~9 Å resolution and pseudoatomic modeling of *Tetrahymena* telomerase, crystal structures of p19 and p45C, and a nuclear magnetic resonance structure of the TER pseudoknot. Two newly identified and functionally distinct RPA-related complexes, Teb1-Teb2-Teb3 (TEB) and p75-p45-p19, are connected to the TERT-TER-p65 catalytic core by p50. The presence of Teb2 and Teb3 is confirmed by mass spectrometry. p19 and p45 are structurally homologous to Ten1 and Stn1, respectively, which are found in the telomere end-binding complex CST (CTC1-STN1-TEN1) that recruits DNA polymerase α . The path of TER on TERT and the location of the TERT essential N-terminal domain (TEN) are revealed, providing mechanistic insights into telomerase function. A network of interactions between TEN, p50, and TEB regulates and enhances activity. p50-Teb1 appears to be structurally and functionally homologous to human TPP1-POT1. Negative-stain electron microscopy of labeled telomeric DNA bound to telomerase reveals the exit path from the template.

CONCLUSION: The structure of the *Tetrahymena* telomerase catalytic core and our identification of telomerase holoenzyme subcomplexes that are homologous to those found at mammalian, plant, and yeast telomeres provide new mechanistic insights and suggest commonalities of telomerase interaction, action, and regulation at telomeres. ■



Two views of the *Tetrahymena* telomerase holoenzyme. The top left image shows a front view of pseudoatomic models as spacefill superimposed on the cryo-EM map. Models of protein domains connected by flexible linkers and not visible in the cryo-EM map are illustrated as ribbons. The bottom right image shows a back-view schematic with the telomerase-bound telomeric G strand connected to a chromosome via double-stranded telomere DNA. Teb1AB domains are presumed to be ordered when DNA bound.

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RESEARCH ARTICLE

STRUCTURAL BIOLOGY

Structure of *Tetrahymena* telomerase reveals previously unknown subunits, functions, and interactions

Jiansen Jiang,^{1,2,3*} Henry Chan,^{1*} Darian D. Cash,¹ Edward J. Miracco,^{1†} Rachel R. Ogorzalek Loo,⁴ Heather E. Upton,⁵ Duilio Cascio,^{1,6} Reid O'Brien Johnson,¹ Kathleen Collins,⁵ Joseph A. Loo,^{1,4,6} Z. Hong Zhou,^{2,3} Juli Feigon^{1,3,6‡}

Telomerase helps maintain telomeres by processive synthesis of telomere repeat DNA at their 3'-ends, using an integral telomerase RNA (TER) and telomerase reverse transcriptase (TERT). We report the cryo-electron microscopy structure of *Tetrahymena* telomerase at ~9 angstrom resolution. In addition to seven known holoenzyme proteins, we identify two additional proteins that form a complex (TEB) with single-stranded telomere DNA-binding protein Teb1, paralogous to heterotrimeric replication protein A (RPA). The p75-p45-p19 subcomplex is identified as another RPA-related complex, CST (CTC1-STN1-TEN1). This study reveals the paths of TER in the TERT-TER-p65 catalytic core and single-stranded DNA exit; extensive subunit interactions of the TERT essential N-terminal domain, p50, and TEB; and other subunit identities and structures, including p19 and p45C crystal structures. Our findings provide structural and mechanistic insights into telomerase holoenzyme function.

Telomerase is a ribonucleoprotein (RNP) complex that extends the telomere DNA at the 3' ends of linear chromosomes, thereby counteracting the loss of DNA from replication and nucleolytic processing (1, 2). Although telomerase is largely inactive in somatic cells, it is active in stem cells and highly active in most cancer cell lines, where its activity is necessary for their immortal phenotype (3–5). Thus, telomerase is an important regulator of aging, tumorigenesis, and stem cell renewal. Telomerase uses a template contained within the integral telomerase RNA (TER) and a telomerase reverse transcriptase (TERT) to synthesize multiple copies of the G-strand telomere repeat (TTGGGG in ciliates and TTAGGG in vertebrates). Telomerase recruitment to telomeres is regulated by the cell cycle, where its activity requires interplay between telomere end-protection and telomerase proteins (6). Telomere end-maintenance also requires coordinated recruitment of telomerase and DNA polymerase α for synthesis of the G and

C strands, respectively (7, 8). In humans, telomerase is recruited to telomeres by components of shelterin (6). Specifically, the TERT essential N-terminal (TEN) domain interacts with TPP1 (9–11), which, in complex with protection of telomeres 1 (POT1), is a processivity factor (6, 12, 13). The budding yeast telomerase holoenzyme subunit Est3 is a structural homolog of the oligosaccharide/oligonucleotide-binding (OB) fold of TPP1 (14) and, like TPP1, interacts with the TEN domain (15). In addition to recruiting telomerase, the human TPP1-POT1 complex recruits the replication protein A (RPA)-like CST (CTC1-STN1-TEN1) complex. RPA binds sequence-nonspecifically to single-stranded DNA (ssDNA) and plays a central role in DNA replication and repair through protein recruitment (16); CST complexes have been proposed as telomere-specific RPAs (17). CST stimulates DNA polymerase α for C-strand synthesis and has other diverse functions in different organisms (8, 18–20). Mammalian CST acts as an inhibitor of telomerase action, determines telomeric 3' overhang structure, and plays broader roles in telomere duplex replication and genome-wide replication restart (8, 18, 20, 21). Budding yeast CST (Cdc13-Stn1-Ten1) subunit Cdc13 recruits the telomerase holoenzyme to telomeres via interaction of Cdc13 with telomerase subunit Est1, associated with Est3 (22–24).

Telomerase can synthesize telomere repeats in vitro with only TERT and TER, but physiological function requires a variety of other proteins (25, 26). Unlike yeast and mammalian telomerase, *Tetrahymena* telomerase is constitutively assembled (27, 28), making it possible to purify and study all

holoenzyme components in a stable complex (29). In addition to TERT and TER, the *Tetrahymena* telomerase holoenzyme contains six other known proteins: p65, p75, p45, p19, p50, and Teb1 (28). TER contains a template/pseudoknot (t/PK) domain, which encloses a template with sequence complementarity to ~1.5 telomere repeats, and a separate activating domain (30). Although TER is essential for activity, the physical arrangement of TER on TERT has remained largely unknown. TERT comprises a telomerase RNA binding domain (TRBD), reverse transcriptase (RT), and C-terminal extension (CTE) that form a TERT “ring” (31), and a separate TEN domain (32) that is important for DNA handling and telomere repeat addition processivity (RAP) (6, 26). p65 binds the TER activating domain [stem-loop 4 (SL4)], inducing a large bend in the RNA that facilitates assembly of TERT with TER to form the RNP catalytic core (33, 34). Teb1 is a paralog of human RPA70, the large subunit of RPA (16, 28). Direct single-stranded telomere DNA binding by Teb1 is necessary for telomerase recruitment to telomeres (27), where it may compete for binding with the *Tetrahymena* telomere end-binding Pot1a (35). p75, p45, and p19 form a ternary complex whose structure and function remain largely unknown (28). The 25 Å resolution negative-stain electron microscopy (EM) structure and individual subunit affinity labeling revealed the overall architecture of *Tetrahymena* telomerase, where TERT occupies the center of the holoenzyme with p65 bound to SL4 below (29). p50, which forms a central hub linking the TERT-TER-p65 catalytic core, p75-p45-p19, and Teb1, can greatly increase RAP and, with Teb1, can dramatically enhance the rate and processivity of long-product synthesis (29, 36). A detailed mechanistic description of telomerase and its interaction at telomeres has been hampered by a lack of structural models, due to difficulties in obtaining samples of sufficient quantity and quality, as well as subunit complexity and flexibility and low sequence identity among subunits from different organisms. Here we report 9.4 and 8.9 Å cryo-electron microscopy (cryo-EM) structures of *Tetrahymena* telomerase holoenzyme. Combining the cryo-EM structures with data from x-ray crystallography, nuclear magnetic resonance (NMR) spectroscopy, negative-stain EM, and mass spectrometry, we discovered two RPA-related complexes: an RPA paralog TEB, comprising Teb1 and previously undetected proteins Teb2 and Teb3, and a CST complex comprising the previously structurally uncharacterized p75-p45-p19. Both are tethered to p50, a potential structural and functional homolog of TPP1, which in turn binds TERT. A pseudoatomic model of the TERT-TER-p65 catalytic core reveals the path of TER on TERT and the location of the TEN domain, and an exit path for the telomeric repeat DNA is proposed, together providing insights into the enzyme mechanism.

Cryo-EM reconstruction and overall structure

Telomerase holoenzyme endogenously assembled in *Tetrahymena thermophila* was affinity-purified

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from a strain bearing a C-terminal 3×Flag (F) and tandem protein A (ZZ) tag on TERT (TERT-FZZ) (29). Cryo-EM specimens of *Tetrahymena* telomerase holoenzyme were prepared using “holey” carbon grids and imaged using a Gatan K2 Summit direct electron detection camera with drift correction (fig. S1). In addition to the preferred front view, particles showed various orientations, which are required for three-dimensional (3D) reconstructions (fig. S2 and methods). Using 40,754 particles, we obtained the intact structure of the *Tetrahymena* telomerase holoenzyme at an overall resolution of 9.4 Å (Fig. 1, A to C, and fig. S1). The negative-stain EM study revealed that the p75-p45-p19 subcomplex is conformationally dynamic (29), which is also apparent in the cryo-EM images (fig. S1, C and D). Therefore, to improve the resolution of the less flexible region, we used a soft mask to exclude the p75-p45-p19 subcomplex from the cryo-EM structure refinement. The resulting reconstruction has an overall resolution of 8.9 Å, with distinguishable secondary structure elements of proteins and RNA (Fig. 1, D to F, and figs. S1 and S3).

Using the features of the secondary structure elements, we were able to rigid-body fit the available atomic-resolution and homology models of protein domains and RNA helical elements (table S1) unambiguously into the 8.9 Å cryo-EM map, except for p75-p45-p19 homologs that were fit into the 9.4 Å cryo-EM map (Fig. 1 and fig. S3). All helical elements of TER—i.e., stem 1 (S1), stem-loop 2 (SL2), SL4, and the pseudoknot (PK)—are

clearly visible with distinct grooves (Fig. 1F), and the path of the single-stranded regions can be approximately traced, although bases cannot be discerned. This allowed us to build a pseudoatomic model of the TERT-TER-p65 catalytic core and Teb1C. The locations of these subunit domains are the same as modeled in the negative-stain EM map within experimental resolution (29), with two exceptions: Teb1C [whose C terminus was correctly localized by Fab labeling (29)] is behind the TEN domain where the PK had been placed, whereas the PK is on the opposite side of TERT by the CTE. In addition, as discussed below, Teb1 forms a heterotrimer with two newly identified proteins, whose location was previously assigned to Teb1C. The p75-p45-p19 ternary complex, whose subunit boundaries could not be determined in the negative-stain EM map (29), is revealed to contain an RPA-like heterotrimer of OB-fold proteins with the domain structure of a CST complex. Overall the pseudoatomic models of the TERT-TER-p65 catalytic core, Teb1-Teb2-Teb3, and p75-p45-p19, which are all linked to p50 in the cryo-EM maps, reveal an intricate network of interactions between the subunits.

The TER t/PK domain encircles the TERT ring

Tetrahymena TERT TRBD-RT-CTE forms a ring-shaped structure and was modeled using the crystal structure of a partial *Tetrahymena* TRBD (residues 259 to 265 and 277 to 519) (37) and a homology model based on the RT-CTE in the *Tribolium* (flour beetle) TERT crystal structure

(Fig. 2A) (31, 38). *Tribolium* TERT lacks the TEN domain, and therefore the position of TEN relative to the TERT ring has remained uncertain. Fitting of the *Tetrahymena* TEN domain crystal structure (32) into the cryo-EM map (Fig. 1F) revealed its location on the active-site side of the TERT ring, stacked over the CTE (Fig. 2A and fig. S4A). Residues 640 to 740 of the insertion in fingers domain (IFD) in the RT [which are not modeled, as the *Tribolium* TERT IFD is much shorter than in *Tetrahymena* and other organisms (39, 40)] appear to be near the TEN domain (Figs. 1B and 2A). The evolutionarily nonconserved sequence (residues 178 to 258) that connects TEN to the TERT ring has no available atomic structure and appears undefined in the cryo-EM maps. Consistent with this lack of a fixed conformation, the TEN domain can be assembled in trans with TER and the TERT ring to form an active catalytic core without linker residues 196 to 215 (41, 42).

Tetrahymena TER comprises a circular t/PK domain, closed by a short S1, that contains the template, the 3'-flanking template recognition element (TRE), the template boundary element (TBE; also called the TERT binding element), SL2, and PK; and an activating domain (SL4), which is connected to S1 by a short single-stranded linker (Fig. 2B) (30). TER was modeled by fitting previously determined (SL2, p65xRRM:S4, L4) and newly determined (PK) (table S4) NMR structures into the cryo-EM density (see methods). The core t/PK domain encircles the TERT ring approximately perpendicularly, such that the template extends across the bottom of the RT domain on one side of the TERT ring, whereas the PK is on the other side next to the CTE (Fig. 2, C to E, and fig. S4). The bottom of S2 is adjacent to the TRBD CP motif, and the 3' end of the TBE is also near the T motif (Fig. 2C and fig. S4), as predicted (29, 37).

The PK, a conserved element of TER that has been proposed to contribute directly to catalysis (43), is on the opposite side of the TERT ring and far from the active site (~20 Å away) (Fig. 2, C and E, and fig. S4A). The PKs of human and yeast TERs contain conserved interactions that stabilize the PK fold through formation of base triples between loops and stems that are important for activity (43–45). The smaller *Tetrahymena* telomerase PK has two base triples in the NMR structure at 10°C and is not stably folded at higher temperatures in the absence of TERT (46). Based on the position of the PK on TERT, we conclude that, rather than contributing directly to catalysis, the correct PK fold is important for proper positioning of TER on TERT. It is plausible that the PK acts like a watchband ratchet clasp during catalytic core assembly, partially or completely unfolding to allow the TER t/PK domain to fit around the TERT ring and then locking onto the TERT ring by folding of the PK. An indirect effect on assembly may explain why mutations that either stabilize or destabilize the PK, at least for human TER, affect telomerase activity in vitro (45).

Outside of the TER t/PK domain, only L4 in S1/SL4 comes into contact with TERT (Fig. 2, C, E, and F;

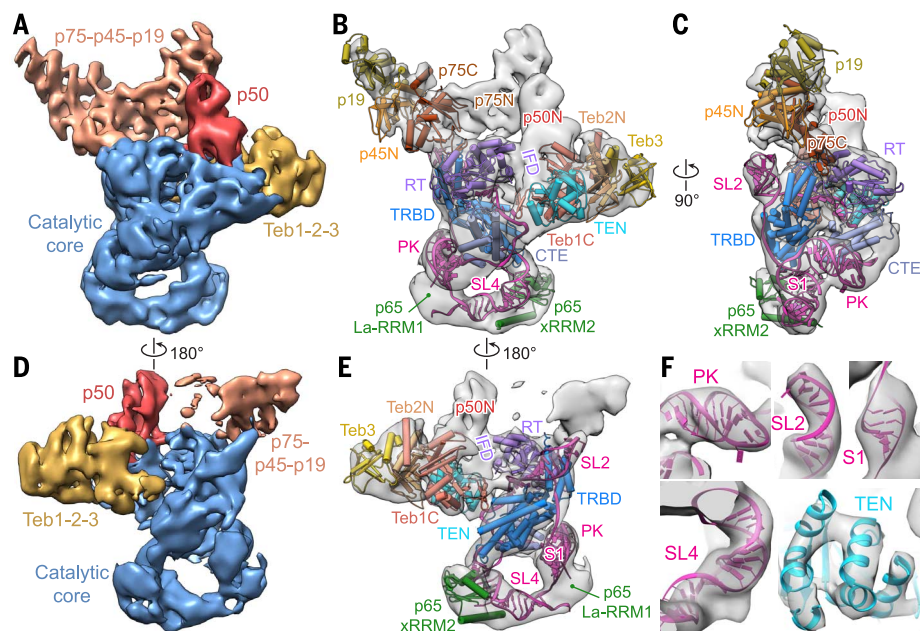


Fig. 1. Cryo-EM reconstructions of *Tetrahymena* telomerase holoenzyme. (A) Front view of the 9.4 Å cryo-EM map, with the catalytic core, Teb1C-Teb2N-Teb3 (TEB), p75C-p45N-p19 (CST), and p50N colored in blue, gold, copper, and red, respectively. (B) Front view of the 9.4 Å cryo-EM map (gray surface) with pseudoatomic models of the TERT-TER-p65 catalytic core, Teb1C-Teb2N-Teb3, and p75C-p45N-p19. (C) Side view of the cryo-EM map and pseudoatomic models shown in (B). (D) Back view of the 8.9 Å cryo-EM map colored as in (A). (E) Back view of the 8.9 Å cryo-EM map, showing pseudoatomic models of TERT-TER-p65 and Teb1C-Teb2N-Teb3. (F) Close-up views of fitting of TER helical domains PK, SL2, S1, and SL4 and TEN domain into the 8.9 Å cryo-EM map. TERT domains are TEN (cyan), TRBD (dark blue), RT (violet, with IFD labeled in violet), and CTE (light blue). Other proteins and TER are colored individually.

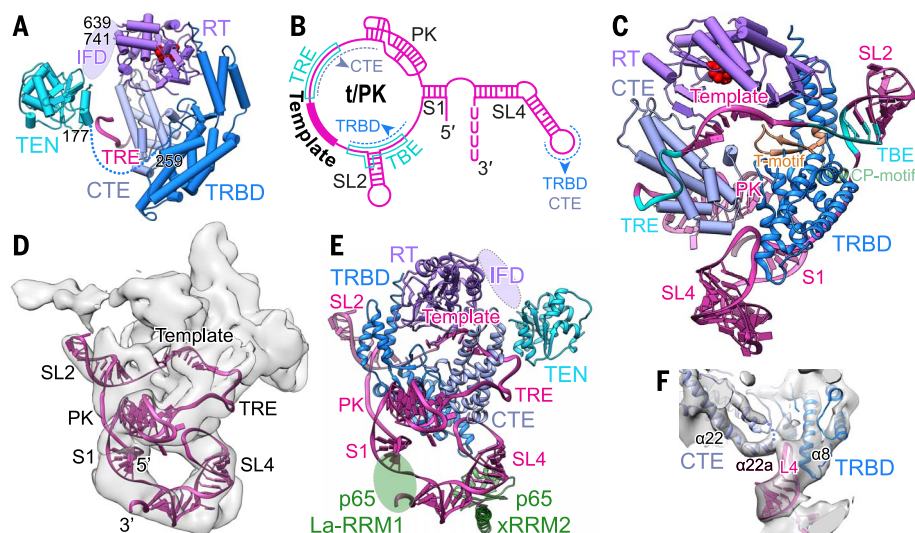


Fig. 2. Structure of the TERT-TER-p65 catalytic core. (A) Model structure of TERT, showing the putative IFD (light violet), active-site catalytic triad residues (red), linker (dotted blue line), and TER TRE. (B) Secondary structure of TER. Locations of CTE and TRBD on TER are indicated with dotted lines. (C) Pseudoatomic model of TERT ring-TER, showing TBE-template-TRE and L4 on TERT, viewed from the active-site side of TERT. (D) Front view of the 8.9 Å cryo-EM map, showing a pseudoatomic model of TERT. (E) Front view of the pseudoatomic model TERT-TER-p65 catalytic core. The putative location of IFD is shown as violet oval. (F) A region of the 8.9 Å cryo-EM map and pseudoatomic model showing L4 at the interface of TRBD (helix $\alpha 8$) and CTE (helix $\alpha 22a$, residues 975 to 983). The numbering of *Tetrahymena* TERT secondary structure elements follows that of the *Tribolium* TERT structure.

and fig. S4A). The p65 La-RRM1 binds the 3' polyU tail (33, 47). La-RRM1 has low resolution in the cryo-EM map, probably due to flexibility or partial disassociation (fig. S1), but appears to be associated with both the 3' tail and the 5' end of S1, thereby possibly linking the 3' and 5' ends of TER (Fig. 2E). p65 C-terminal xRRM2 (33) binds to and bends S4, inserting L4 between the end of TRBD helix $\alpha 8$ and a short helix from the CTE ($\alpha 22a$) that does not exist in *Tribolium* TERT but has well-defined density in the 8.9 Å cryo-EM map (Fig. 2F). In complex with p65, SL4 stimulates activity, hierarchical assembly, and holoenzyme stability (33, 34, 47, 48). L4 binds with high affinity to the TRBD, but its specificity for the CTE remains unknown. A hypothesis consistent with the above, as well as the location of L4 far from the active site and PK, proposes that L4 stabilizes a closed conformation of the TERT ring via specific interactions with TRBD and CTE.

The TER single-stranded region containing TRE, the template, and 3'TBE spans the active-site side of the TERT ring between the CTE and the TEN domain, across the RT, and over the TRBD T/CP pocket, respectively (Fig. 2C). The cryo-EM structure is of telomerase in the apo state before binding telomeric ssDNA. The TRE nucleotides, on the 3' side of the template, pass between the CTE and the TEN domain and could contact the TEN domain. There is clear cryo-EM density for the 3' half of the template only (fig. S3E), suggesting flexible positioning of the 5' residues close to the TBE. Based on the distance from TER S2, the template appears to be positioned near where it would be at the end of telomere repeat synthesis—i.e., the 5' end of the template

is closest to the active site. The template also appears to be displaced by ~ 7 Å from its position at the active site in the crystal structure of *Tribolium* TERT in complex with an RNA-DNA hybrid helix (38), as proposed for the strand-separation step (49). The position of S2/TBE on TERT relative to the 5' end of the template suggests a structural mechanism for template boundary definition. The single-stranded residues of the TBE, which flank S2, wrap on either side of the TERT ring (Fig. 2C and fig. S4B). At the end of telomere repeat synthesis, the S2 and TBE-TRBD interactions could act as an anchor to prevent residues beyond the template from being pulled into the active site (Fig. 2C and fig. S4B).

Teb1 forms an RPA-like complex with two newly identified proteins

Although Teb1 has four OB-fold domains (N, A, B, and C) (50), only the Teb1C domain and, in some cases, very weak density for Teb1B were apparently visible in the negative-stain EM class averages of *Tetrahymena* telomerase (29). Fitting of Teb1C into the cryo-EM map revealed density of unknown origin in the “knob” next to Teb1C (Fig. 3 and fig. S5A). An exhaustive analysis of the potential positions and fittings of other holoenzyme proteins revealed no known candidates for the knob density (fig. S5A). Because Teb1 is an RPA70 paralog, we investigated whether this part of the cryo-EM map might contain paralogs of the other two RPA subunits (RPA32 and RPA14). We found that the crystal structure of the RPA heterotrimeric core complex (RPA70C-RPA32N-RPA14) (51) fit particularly well into the cryo-EM map, with RPA70C positioned in the cryo-EM density

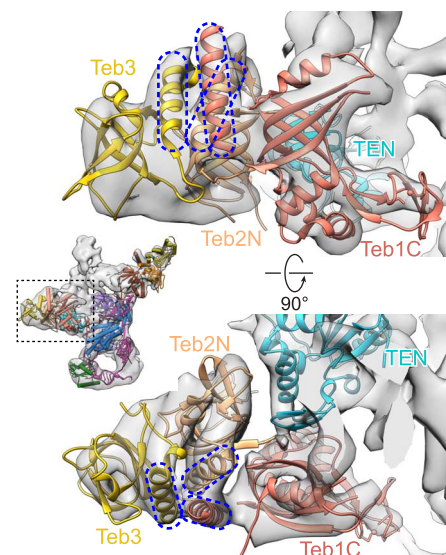


Fig. 3. Identification of two previously unknown holoenzyme proteins, Teb2 and Teb3. Shown are two views of the knob and Teb1C region of the 8.9 Å cryo-EM map with a model of Teb1C-Teb2N-Teb3 based on fitting of RPA70C-RPA32N-RPA14 into the cryo-EM map, followed by replacement of RPA70C with Teb1C, except for the RPA70C C-terminal α helix. The TEN domain is shown in cyan. The three-helix bundle between the C-terminal residues of Teb1C, Teb2N, and Teb3 (modeled from RPA) is highlighted with dashed lines. The inset shows the corresponding back view of the holoenzyme for reference.

for Teb1C and the RPA32N and RPA14 OB folds in the knob (Fig. 3). RPA trimerization requires the formation of a three- α -helix bundle from the C-termini of RPA70, RPA14, and RPA32N. The predicted C-terminal α helix of Teb1C is disordered in the crystal structure (50). However, in the cryo-EM map there is clear density of three α helices involved in the trimerization, with one of them near the C terminus of Teb1C, which we modeled in from the RPA70 structure (Fig. 3 and fig. S3H).

To confirm the presence of putative Teb1 heterotrimer proteins in the holoenzyme, we used liquid chromatography–tandem mass spectrometry (LC-MS/MS) to analyze telomerase purified from the TERT-FZZ strain. All seven of the known *Tetrahymena* telomerase proteins, plus two additional hypothetical proteins (TTHERM_001113129 and TTHERM_00439320) (www.ciliate.org), were detected with high confidence (table S2A). PSI-BLAST (Position-Specific Iterated Basic Local Alignment Search Tool) (52) searches of corrected cDNA sequences of these 31- and 14-kD proteins showed homology with predicted RPA32 and RPA14 homologs, respectively. Secondary structure predictions by Jpred4 (53) are consistent with an OB fold for the 14-kD protein and an N-terminal OB fold and C-terminal winged-helix (WH) domain for the 31-kD protein. Multiple sequence alignments of these domains with RPA32 and RPA14 orthologs show high similarity (fig. S6). We previously observed that telomerase purified from

a *Tetrahymena* strain containing N-terminally ZZP-tagged p50 (F-p50 telomerase) lacks the knob (fig. S5B) and has low RAP, which was attributed to the loss of Teb1 induced by the ZZP-tag on p50 (29). We analyzed F-p50 telomerase by LC-MS/MS and found that the two newly identified proteins and Teb1 are absent (table S2B), which confirms that these two additional proteins are located at the knob in the cryo-EM map and form a complex with Teb1. We conclude that there are two previously undetected proteins in the *Tetrahymena* telomerase holoenzyme that form a heterotrimer with Teb1, here named TEB, paralogous to the ssDNA binding RPA, except specific for telomeric G-strand DNA. In *Tetrahymena*, only the large subunit of RPA, Rfa1, has been identified (54). Transcript expression levels of the two newly discovered Teb1-binding proteins, here named Teb2 and Teb3, are much higher than other telomerase proteins and more similar to the level of Rfa1, as judged by expressed sequence tag abundance (55). These observations suggest that these two subunits may be shared between *Tetrahymena* RPA and TEB.

Multiple interactions between TEN, TEB, and p50 regulate telomerase activity

The TEN domain has been identified as a major determinant of telomerase activity (6, 42). In addition to its potential TRE interaction (Fig. 2), the cryo-EM map reveals that TEN is adjacent to p50, Teb1C, Teb2N, and the IFD (Fig. 4, A and B). TEN, Teb1C, and p50 contact each other in a triangular arrangement. The Teb1C-TEN interaction is clearly defined in the pseudoatomic models (Fig. 4C). A Teb1 Phe⁵⁹⁰→Ala⁵⁹⁰ (F590A)/F648A double mutant was previously shown to ablate purification of telomerase by Teb1-FZZ expressed in cells (27). These two residues of Teb1 are on the Teb1C-TEN interface (Fig. 4C), accounting for the loss of function. In the cryo-EM map, the density of TEN is also in contact with that of Teb2. TEN residues 77 to 87, which are missing in the crystal structure due to disorder (32), might form a structured interface with Teb2N in the holoenzyme (Fig. 4D). In vitro telomerase reconstitution activity assays, which lacked Teb2 and Teb3, showed that deletion of the Teb1C putative C-terminal α helix (Δ CT α H) had little effect on activity (27, 54). However, purification of Teb1(Δ CT α H)-FZZ expressed in vivo did not recover any telomerase activity or holoenzyme subunits, indicating no in vivo telomerase assembly with Teb1(Δ CT α H) (27). Thus, Teb2 and Teb3, which interact with the Teb1 C-terminal α helix, likely stabilize association of Teb1 with telomerase.

To provide biochemical evidence for these interactions, we used in vitro reconstitution of complexes with individual TEB subunits expressed in rabbit reticulocyte lysate (RRL) to investigate whether Teb2-Teb3 had any effect on telomerase activity (Fig. 4E). Our activity assays were designed to be sensitive to improved holoenzyme assembly of Teb1 by the use of a very low level of Teb1 expressed in RRL, rather than a saturating concentration of purified bacterially expressed protein,

and by purification of RNP from unbound Teb1 before the activity assay. Addition of Teb1 to the catalytic core plus p50 greatly increases RAP, as expected from previous studies (29, 36). Coexpression of Teb2-Teb3 with Teb1 further increased overall activity, consistent with additional stabilization from synergistic interaction of Teb2 with the TEN domain and Teb1C. Addition of Teb2-Teb3 alone, without Teb1, provided no activity enhancement. Together, the cryo-EM structure, activity assays, and in vivo functional studies (27) discussed above support the notion that the TEN-TEB protein interaction network has crucial physiological importance.

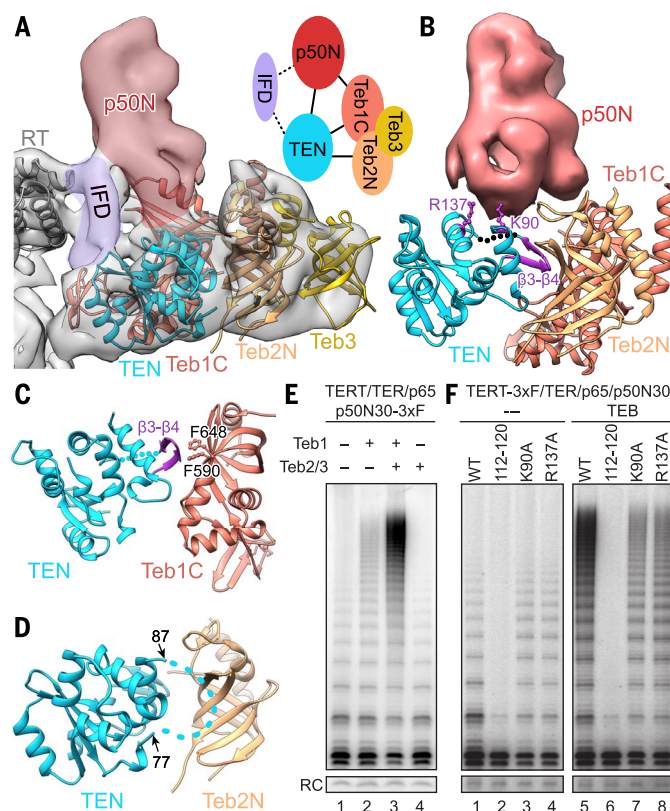
The cryo-EM density of p50N appears to contact the TEN domain, as well as Teb1C, the IFD, and p75-p45-p19 (Figs. 1, B and E, and 4A). p50, which has no known sequence or structural homology to other proteins, has a 30-kD N-terminal domain (p50N) that is required for the high level of processive repeat synthesis conferred by p50 binding to the catalytic core (36). Only p50N is apparently visible in the class averages and 3D reconstructions of negative-stain EM images (36) and also in the cryo-EM maps. Like TPP1, p50 contacts the TEN domain (Fig. 4, A and B), and

like TPP1-POT1 (13), p50-Teb1 greatly increases RAP (36). These data suggest that p50 could be a structural and functional ortholog to vertebrate TPP1. Although TPP1 was initially identified as a telomere binding protein complex with POT1 bound to the G-strand overhang to block telomerase access, it has emerged as also being the direct mediator of telomerase recruitment, activation, and homeostasis set-point regulation (6, 7, 56, 57). We fit the structure of the TPP1 OB fold into the 8.9 Å cryo-EM map, but only the characteristic OB-fold β barrel matches well to the cryo-EM density (fig. S3, M to O). This is not surprising, due to the expected cofolding of p50 loops and helices with its interaction partners. Though the fitting does not provide definitive structural homology to TPP1, it does provide evidence that p50N is an OB fold.

The TEN domain has an extensive interface with p50 that apparently includes residues 122 to 127, which are missing in the crystal structure due to disorder (32) (Fig. 4B) and may cofold with p50. We tested whether other TEN domain interactions with p50 inferred from the pseudoatomic model and cryo-EM map were important

Fig. 4. Subunit interactions between p50, TEN, IFD, Teb1C, Teb2N, and Teb3. (A) Region of the 8.9 Å cryo-EM map showing density linking p50N, TEN, IFD, and TEB. The inset shows a schematic of the interactions. Dotted lines represent inferred interactions from the cryo-EM density and atomic models fitted to the cryo-EM map. (B)

Interactions between p50N (red, cryo-EM density), TEN, Teb1C, and Teb2N. The TEN residue K90 side chain corresponding to the human TEN domain residue K78 that interacts with TPP1, R137, and the β 3- β 4 hairpin (residues 112 to 120) is highlighted in violet. (C) Interactions between TEN and Teb1C. Teb1C F590/F648 residues (orange stick) that together abrogate the Teb1C-TEN interaction and the TEN β 3- β 4 hairpin (violet) are indicated. TEN residues 122 to 127, disordered in the crystal structure, are represented by dotted lines in (B) and (C). (D) Interactions between TEN and Teb2N. TEN residues 77 to 87, disordered in the crystal structure, are shown as a dashed line. Pseudoatomic models of TEN, Teb1C, and Teb2N interactions are based on crystal structures of TEN, Teb1C, and RPA32N-RPA14 fit into the 8.9 Å cryo-EM map. (E and F) In vitro reconstitution telomerase activity assays for the effect of (E) TEB proteins and (F) TEN domain mutations on the catalytic core assembled with p50N30. TEN domain mutations were tested without (lanes 1 to 4) and with (lanes 5 to 8) TEB. WT, wild type; RC, recovery control.



for RNP activity stimulation by p50, in a manner similar to TEN interactions with TPP1 and Est3. A cluster of residues on human TPP1 (and Est3), called the TEL patch, has been identified as the surface of interaction with TEN (9–11, 14, 56, 57). This interaction is essential for human telomerase recruitment to telomeres. Human TEN residues whose substitution disrupts the interaction of telomerase with TPP1 without greatly affecting catalytic activity have been identified; among these, a direct interaction between Lys⁷⁸ (K78) and TPP1 has been demonstrated (57). The equivalent *Tetrahymena* TEN residue based on homology modeling of human TEN with the crystal structure of *Tetrahymena* TEN is K90, which is located near the density for p50 in the cryo-EM map (Fig. 4B). We used our *in vitro* telomerase reconstitution activity assay to investigate whether K90A at the putative TEN-p50 TEL patch interface imposes a defect in p50 stimulation of telomerase activity (Fig. 4F). K90A had a modest but notable effect on overall activity. The same results were obtained for TEN R137A (R, Arg), which is also located at the TEN-p50 interface in the cryo-EM map. These results support similar interactions between *Tetrahymena* TEN-p50 and human TEN-TPP1. TEN K90A and R137A did not abrogate TEB stimulation of high RAP (Fig. 4F), consistent with direct TEN domain interactions with TEB and p50-bridged TERT-TEB association. TEN residues 112 to 120 form a β hairpin, exclusive to *Tetrahymena* TEN, that inserts at the interface be-

tween p50, Teb1C, and Teb2N (Fig. 4B). An adjacent block sequence substitution TEN_(108–113)NAAIRS (N, Asn; I, Ile; S, Ser) abolishes p50 binding (42). In our experiment, we replaced the β hairpin with GSSG (G, Gly). This substitution abolishes p50 activity stimulation, even with added TEB, which is consistent with disruption of both p50 and TEB interactions, but did not affect catalytic core activity. Taken together, these results validate the network of TEN-p50-Teb1C-Teb2N interactions proposed based on the cryo-EM structure (Fig. 4, A to D) and provide supporting evidence that p50 is a structural and functional paralog of TPP1.

The cryo-EM density that we attribute to the IFD appears to independently contact p50 and the TEN domain N terminus, which is disordered in the crystal structure (32) (Figs. 1B and 4A). The IFD is exclusive to TERTs and is important for the translocation step required for RAP (39, 40). We suggest that the human TERT IFD may have parallel, as yet undetected, interactions with the TPP1 OB fold and TEN domains.

p75-p45-p19 is a CST complex

We determined a 2.3 Å x-ray crystal structure of p19 (table S3), which revealed an OB fold most structurally homologous to human Ten1 (Fig. 5A and fig. S7), suggesting the possibility that p75-p45-p19 might be a CST- or second RPA-like complex. We found that human RPA70C-RPA32N-RPA14, which fit the cryo-EM density of TEB as described above, also fit equally well in the density at the

“tip” of the p75-p45-p19 subcomplex in the 9.4 Å cryo-EM map (Fig. 5B). For our model, RPA14 was then replaced with the p19 crystal structure, except for its unstructured C terminus, which forms an α helix in a three-helix bundle with the other two proteins in the RPA complex (Fig. 5B). p19 α 2 and α 3, which are lacking in RPA14, fit well into the cryo-EM density at the very end of the tip.

The positions of the C-terminal α helices of RPA70C and RPA14 correspond to the locations of the C termini of p75 and p19 determined by Fab labeling in negative-stain EM (Fig. 5B) (29). We used copurification and limited proteolysis to establish that p45 is composed of an N-terminal domain (p45N) that binds p19 and an independently folded C-terminal domain (p45C) (see methods). These results are consistent with the hypothesis that p45 is a Stn1 or RPA32 homolog. Previous attempts to locate the position of p45 in the holoenzyme by Fab labeling of its C terminus were unsuccessful, as Fab was apparently not visible in negative-stain EM class averages of telomerase holoenzyme (29). Based on the above, we hypothesized that p45C might be connected to p45N in the p75-p45-p19 subcomplex via a flexible linker, and thus Fab-bound p45C was not visible in holoenzyme class averages due to positional flexibility relative to the holoenzyme. We screened >2000 negative-stain EM particles of Fab-labeled p45-F telomerase. The majority had a cluster of three Fab (the 3 $\times$ FLAG tag can bind up to three Fab) located in various positions ~100 Å from the location of p45N (Fig. 5D and fig. S8). The C-terminal domains of RPA32 and Stn1 are composed of WH and tandem WH (WH1-WH2) domains, respectively (17, 19, 58, 59). We determined a 2.4 Å crystal structure of p45C (table S3) and found that it comprises the WH1-WH2 domains (Fig. 5C). The OB fold followed by two WH domains suggests that p45 is a Stn1 homolog (17, 19, 58, 59). Structural alignments (fig. S7), sequence alignments (fig. S6), and phylogenetic cluster analysis (fig. S9) of p19 and p45 domains also support their identification as Ten1 and Stn1 orthologs, despite low sequence homology of CST proteins between kingdoms. In the 9.4 Å cryo-EM map, the remaining unmodeled density in the p75-p45-p19 complex, which appears to contain at least two additional OB folds and contacts p50, can only be attributed to p75 (Fig. 5B). Consistent with this observation, among p75-p45-p19, p75 is necessary and sufficient to bind p50 *in vitro* (36). There are distinct differences between p75 and Teb1 in terms of their interaction with p50 and their effect on telomerase activity. The cryo-EM maps show that p75 contacts p50 with a domain near its N terminus; in contrast, Teb1 binds p50 and the TEN domain with its C-terminal OB fold. How the potential p75 ortholog CTC1 interacts with telomerase is unknown, but it is interesting to note that the functionally divergent Cdc13 binds yeast telomerase holoenzyme protein Est1 through a recruitment domain adjacent to its N-terminal OB fold (8, 17, 24). *In vitro* reconstitution assays show that the addition of p75 (or p75-p45-p19) to the catalytic core plus p50 stimulates activity slightly but without an increase in

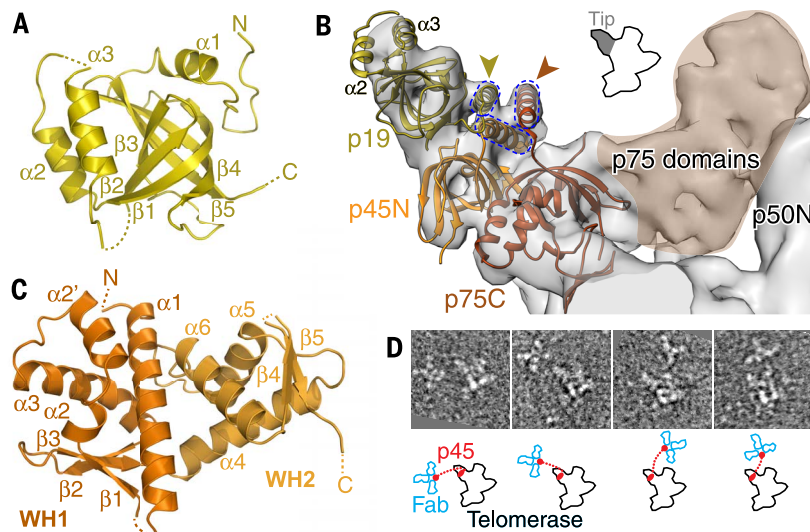


Fig. 5. Identification of p75-p45-p19 as a *Tetrahymena* CST complex. (A) Crystal structure of p19, an OB fold. (B) Model of p75C-p45N-p19 based on fitting of RPA70C-RPA32N-RPA14 into the 9.4 Å cryo-EM map, followed by replacement of RPA14 by p19, except for the RPA14 C-terminal helix. p19 α 2 and α 3 account for the density at the end of the tip. Gold and orange arrowheads point to the locations of the p19 and p75 C termini, respectively, previously determined by Fab labeling in negative-stain EM (29). The three-helix bundle between the C-terminal residues of p75, p45N, and p19 (modeled from RPA) is illustrated with dashed lines. (C) Crystal structure of p45C, a WH-WH domain. β 5 is domain-swapped from a neighboring protein in the crystal lattice. (D) (Top) Negative-stain EM images of four typical p45-Fab-labeled telomerase holoenzyme particles, showing a cluster of 3 Fab bound to the C terminus of p45 at various locations near the holoenzyme. The side length of each image box is 44 nm. (Bottom) Corresponding outlines of telomerase and Fabs are shown in black and blue, respectively. Red dots indicate the p45C (attached to 3 Fab) and p45N (on telomerase) domains; dotted lines represent the linker.

RAP; in contrast, Teb1-p50 interaction stimulates high RAP (29, 36). We conclude that p75-p45-p19 is structurally and functionally distinct from TEB and is most similar to a CST complex. CST has been identified in yeasts, plants, and mammals (8, 19); this is the first evidence for the presence of CST in ciliates.

The telomeric DNA exits the template toward Teb1C

To obtain information on the path of telomeric DNA on telomerase, we prepared a holoenzyme bound to a short telomeric DNA, biotin-5'-d (GTTGGG)₂GT_LT_LGT_LG_LG, where T_L and G_L are locked nucleic acid (LNA) nucleotides (60). For this DNA, six nucleotides (nts) should bind the template and 13 nts should extend out from its 3' end. We then bound biotin with streptavidin, linking two telomerase holoenzymes together via the biotin binding sites in each streptavidin tetramer. Visualization of these dimers of the telomerase holoenzyme by negative-stain EM confirms that the telomeric DNA is bound (Fig. 6, A to C). The class averages and 3D reconstructions show clearly visible density of streptavidin located near Teb1C-Teb2N and the putative location of Teb1B (29) on the backside of the telomerase holoenzyme (Fig. 6, B to D). These results reveal that telomeric DNA exits the template from the backside of telomerase and toward Teb1C (Fig. 6D). Furthermore, within the resolution (~30 Å) of negative-stain EM, there is no large-scale structural rearrangement of TERT between the apo structure without DNA and the structure with telomeric DNA bound to the template, consistent with the crystal structure of *Tribolium* TERT without TER (31) versus with an RNA-DNA hairpin mimicking a template-DNA hybrid (38).

Implications for telomerase catalytic activity and association with telomeres

The cryo-EM structures, p45C and p19 crystal structures, PK NMR structure, domain modeling, and mass spectrometry data presented here reveal a complex RNP composed of three ternary complexes tethered by p50: a TERT-TER-p65 catalytic core; an RPA analog TEB, comprising Teb1-Teb2-Teb3; and a *Tetrahymena* CST complex comprising p75-p45-p19. TER wraps around the TERT ring and interacts with all four domains of TERT. The TEN domain is close to TER, the TERT ring IFD, p50, Teb1, and Teb2, signaling its importance in the catalytic cycle of telomerase. In addition to the protein domains visible in the cryo-EM map, there are several domains that are not observed due to flexible positioning or intrinsic disorder in the absence of binding partners; these are Teb1 N, A, and B; putative Teb2C, p45C, p50C, p65N; and possibly p75N (schematized in Fig. 7A). Teb1A and Teb1B bind telomere DNA (54), whereas the others (except for p65N) may recruit proteins involved in C-strand synthesis, nucleolytic end-processing, unwinding of G quadruplexes, telomere end-binding, and DNA repair, thereby coordinating telomere maintenance.

In the catalytic core, the TER interactions with TERT are exclusively from the t/PK domain and

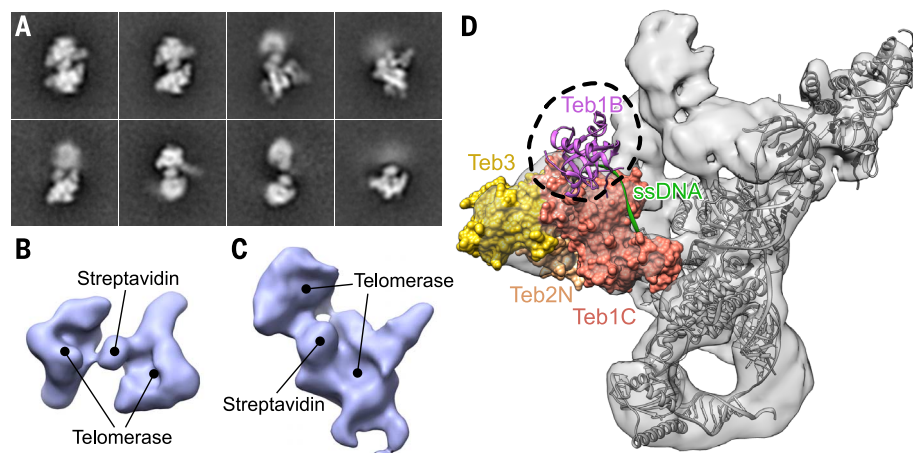


Fig. 6. Telomere ssDNA exits from the backside of the *Tetrahymena* telomerase holoenzyme.

(A) Negative-stain EM class averages of the telomerase holoenzyme dimerized by primer-biotin-streptavidin-biotin-primer. The side length of each image box is 42 nm. (B and C) Random conical tilt (RCT) reconstruction of dimeric telomerase holoenzymes. One of two telomerase holoenzymes in (C) shows uninterpretable features due to flexible positioning of the two holoenzymes relative to each other. (D) A 9.4 Å cryo-EM map of the telomerase holoenzyme (gray surface) and the position of primer-attached streptavidin (black dashed circle), as identified by negative-stain EM RCT reconstruction in (B) and (C). Teb1B (purple) and ssDNA (green) exiting through Teb1C are modeled by fitting the crystal structure of the RPA:ssDNA complex (PDB ID 4GNX).

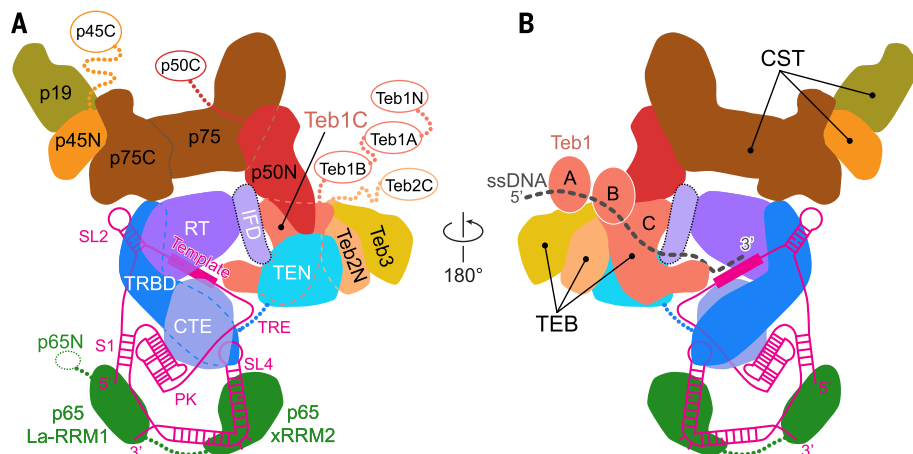


Fig. 7. Schematics of the complete *Tetrahymena* telomerase holoenzyme and DNA exit path.

(A) Arrangement of the subunits and domains of the *Tetrahymena* telomerase catalytic core, TEB, and CST complexes tethered to p50, shown as front view. Domains connected by flexible linkers and not seen in the cryo-EM map are shown with oval outlines. (B) Arrangement of the holoenzyme with proposed path of telomere ssDNA, shown as back view. Teb1AB domains are presumed to be ordered when DNA-bound.

L4. Because a t/PK and a stem terminus element like L4 are almost universally found in other organisms (2, 30) and TERT is highly conserved, *Tetrahymena* telomerase provides general insights into TER-TERT interactions and assembly. Human TER has a much larger pseudoknot, but the region containing the tertiary stem-loop interactions is comparable in size and probably interacts with TERT at the same location near the CTE, and the human TER t/PK could similarly encircle the TERT ring. This would place the other end of the pseudoknot close to the TEN domain, as implicated biochemically (41). Vertebrate telomerase contains a conserved three-way junction, the CR4-CR5 domain, that binds the TRBD (61) and

has a stem-loop (P6.1) that is critical for activity (62). The P6.1 loop probably inserts at the TRBD-CTE interface (61, 63), similarly to *Tetrahymena* TER L4.

In the cryo-EM structure of apo telomerase presented here, the template is apparently positioned with the 5' end near the active site. This places the 3' end of the template close to Teb1C, where the telomeric DNA binds as it exits the holoenzyme. We suggest that this would facilitate (re)binding of the primer to the template 3' end before translocation. Based on homology with the crystal structures of the RPA:ssDNA complex (51), a model of Teb1AB bound to ssDNA based on the Pot1AB:ssDNA complex (50), and the position

of the 5' end of telomeric DNA on telomerase revealed by streptavidin labeling, we can model a possible path of the telomere DNA on telomerase (Fig. 7B). Although the DNA is shown going straight from the template to Teb1, it is possible that during the catalytic cycle the DNA strand may interact with TEN, TRBD, and/or CTE. Within the catalytic core, the TEN domain has been implicated to play a role in ssDNA handling and act as an anchor site (32, 64) and/or in active-site use of the DNA–template RNA hybrid (41). The TEN domain is positioned so that the exiting end of the short DNA–template helix could contact its (unmodeled) N and C termini at the end of a complete telomere repeat synthesis, where this interaction could facilitate template DNA strand separation and help direct the DNA toward Teb1 (fig. S10).

The identification of two RPA related complexes in the *Tetrahymena* telomerase holoenzyme and their constitutive rather than cell-cycle-regulated association allows us to use studies of the *Tetrahymena* telomerase holoenzyme to inform models for interaction and function of human TPP1-POT1 and CST. Like p50-TEN, the interaction between human TPP1 and TEN is essential for bridging telomerase to telomeres (9, 11, 56, 57). Although Teb1C is homologous to RPA70C, Teb1AB is more similar to POT1 (50), which suggests that TEB functions to temporarily prevent rebinding of the telomere-bound Tpt1-Pot1a complex (65). Consistent with the conformational flexibility of p75-p45-p19 around p50 (29), this CST complex could bind the telomeric DNA as it exits Teb1 to recruit DNA polymerase α for coordinated C-strand synthesis. In summary, the structure of the *Tetrahymena* telomerase catalytic core and identification of telomerase holoenzyme subcomplexes homologous to those found at mammalian, plant, and yeast telomeres provide new mechanistic insights and suggest commonalities of telomerase interaction, action, and regulation at telomeres.

Materials and methods

EM specimen preparation and data collection

Tetrahymena telomerase holoenzyme was purified following the previously described protocol (29), with some modifications. Briefly, 12 liters of cell culture of TERT-FZZ strain was used for tandem affinity purification following the established procedures (29). The final Flag elution was effected by incubating the telomerase-bound anti-Flag M2 affinity gel with 1.5 ml of elution buffer (20 mM HEPES-NaOH at pH 8.0, 50 mM NaCl, 1 mM MgCl₂, 1 mM TCEP-HCl, 0.025% IGEPAL CA-630, and 200 ng/ μ l of 3 $\times$ Flag peptide) at 4°C. The eluate was incubated with 30 mg of Bio-Beads SM-2 absorbent (Bio-Rad) at 4°C for 2 hours by end-over-end rotation. The supernatant was then concentrated to 30 μ l using a Microcon YM-10 centrifugal filter (Millipore).

For cryo-EM, 2.5 μ l of the sample was applied to a glow-discharged Quantifoil R2/1 grid. The grid was blotted with filter paper to remove excess sample and was flash-frozen in liquid ethane with FEI Vitrobot Mark IV. The frozen-hydrated

grids were loaded into an FEI Titan Krios electron microscope operated at 300 kV for automated image acquisition with Leginon (66). Micrographs were acquired with a Gatan K2 Summit direct electron detection camera operated in the electron-counting mode at a calibrated magnification of 36,764 $\times$ (pixel size of 1.36 Å on the sample level) and defocus values ranging from -2.0 to -7.0 μ m. A GIF Quantum LS Imaging Filter (Gatan) was installed between the electron microscope and the K2 camera, but the energy filter (slit) was not used. The dose rate on the camera was set to ~ 8 electrons (e^-) per pixel per second, and the total exposure time was 12 s fractionated into 48 frames of images with a 0.25-s exposure time for each frame. Frame images were aligned and averaged for correction of beam-induced drift using the graphics processing unit-accelerated motion correction program (67). The average images from all frames were used for defocus determination and particle picking, and those from the first 28 frames (corresponding to $\sim 30 e^-/\text{Å}^2$ of total dose on sample) were used for further data processing, including image classification and 3D structure refinement. A total of 4210 micrographs were used in the data processing.

Image processing

The defocus value of each cryo-EM micrograph was determined by CTFFIND (68), and the micrographs were corrected for contrast transfer function (CTF) by phase-flipping with the corresponding defocus and astigmatism values using Bsoft (69). A total of 478,698 particles were automatically picked using DoGpicker (70) and windowed out in dimensions of 256 pixels by 256 pixels using batchboxer in EMAN (71). The particles were binned to dimensions of 128 pixels by 128 pixels (pixel size of 2.72 Å) before the following processing. The binned particles were subjected to 2D and 3D classifications by RELION (72), following the recommended procedure (www2.mrc-lmb.cam.ac.uk/relion/). We performed two consecutive rounds of 2D classifications; in each round, the particles were classified into 100 classes for 25 iterations. After each round of 2D classification, class averages were visually inspected, and particles in the “bad” classes (i.e., those with fuzzy or uninterpretable features or fuzzy density of the p75-p45-p19 subcomplex) (fig. S1B) were removed. About 20% of the class averages were kept after each round of 2D classification, and 47,251 particles were selected for the following 3D classification. The previously obtained negative-stain EM reconstruction at 25 Å resolution (29) was low-pass filtered to 60 Å to serve as the initial model for 3D classification. The 3D classification generated five classes (fig. S1D), four of which showed an intact holoenzyme structure and were combined into a data set of 40,754 particles for 3D autorefinements by RELION as follows: First, a 3D autorefinement was performed using a spherical mask. The resolution was estimated to be 9.4 Å by the *reliion_postprocess* program using the “gold-standard” Fourier shell correlation at a 0.143 criterion, with a soft mask for which the masking effect was corrected by phase randomization. Second, the density corresponding

to the flexible p75-p45-p19 subcomplex was removed from the 9.4 Å cryo-EM map using the “Volume Erase” tool in UCSF Chimera (73), and the resulting map was used to generate a soft-edge mask by the *reliion_mask_create* program. This soft-edge mask was then used in the other 3D autorefinement, in which the p75-p45-p19 subcomplex was excluded during the structure refinement and only included in the final reconstruction. The resolution of this focused refinement was 8.9 Å, estimated as described above. The cryo-EM maps were sharpened with B-factor and low-pass filtered to the stated resolution using the *reliion_postprocess* program. The local resolution was calculated with ResMap (74), using two cryo-EM maps independently refined from halves of data.

Tetrahymena telomerase holoenzyme particles adopted a preferred front view in negative-stain EM grids (29) or in cryo-EM Quantifoil grids with holey carbon or coated with continuous carbon film, probably due to its irregular and flattened shape. We tried numerous sample and cryo-EM grid conditions to overcome this preferred orientation problem. The current protocol involving Bio-Beads had the most improved effect on the orientation distribution. However, $\sim 60\%$ of the particles were still in the front view configuration in the cryo-EM images. To assess the effect of preferred orientation on reconstruction resolution, the particles were divided into halves (“uniform” and “preferred”) that showed more uniform or more preferred orientation distribution, respectively (fig. S2). Briefly, the particle orientation file (the “data.star” file generated by RELION 3D autorefinement) of 40,754 particles was analyzed to group particles with the same orientation (same AngleRot and AngleTilt). A threshold of particle count was set so that the particles above the threshold in each orientation group (where the particle count in that group was larger than the threshold) were sent to the preferred half, and the rest were kept in the uniform half. Separation of particles within an orientation group was random. These two halves of the data set were then subjected to 3D autorefinement, using the same parameters as those for the 8.9 Å reconstruction. Comparisons of orientation distributions and 3D reconstructions show that the uniform half had sufficient orientations to obtain a high-quality 3D reconstruction at an overall resolution better than 9.3 Å, and addition of the preferred half only improved the resolution modestly (fig. S2).

Fitting of atomic models into the cryo-EM maps

For the pseudoatomic model of TERT-TER-p65 and Teb1C, the x-ray crystal and NMR structures of *Tetrahymena* TERT TRBD [Protein Data Bank identification code (PDB ID) 2R4G] (37), TERT TEN (PDB ID 2B2A) (32), Teb1C (PDB ID 3U50) (50), TER SL2 (PDB ID 2FRL) (29), the model of p65 xRRM2:SL4 (33), and the homology model of *Tetrahymena* TERT RT-CTE domains based on *Tribolium* TERT (PDB IDs 3DU6 and 3KYL; TERT CTE residues 1058 to 1117 were not modeled due to low sequence homology to *Tribolium* TERT) (29, 31, 38)—which were previously assembled

together by fitting into the negative-stain EM 3D reconstruction (29)—were placed as a whole in an approximate position in the cryo-EM maps, followed by fitting the individual domain structures into the cryo-EM map using the “Fit in Map” function in UCSF Chimera (73). These domain structures or models fit into the cryo-EM map with subtle rotations and translocations, except for Teb1C, which was moved to the cryo-EM density behind TEN and next to TRBD and matched this with high consistency. The PK structure (PDB ID 2N6Q) was manually placed into the cryo-EM density matching its size and shape, as identified by exhaustive visual inspection of the cryo-EM map, and then fit into the density using UCSF Chimera. S1 was modeled as an ideal A-form helix and fit into cryo-EM density that matched its shape near the PK and SL4. The template was located by fitting the crystal structure of *Tribolium* TERT in complex with an RNA-DNA hybrid helix (PDB ID 3KYL) (38) into the cryo-EM map and finding unmodeled density near the RNA strand. The density assigned for the template is displaced by ~7 Å relative to that in the fitted *Tribolium* TERT. The remaining single-stranded regions of TER connecting the above fitted structure elements were modeled into the cryo-EM densities using Coot (75) as follows: First, ideal A-form single-stranded RNA fragments were approximately placed between the flanking high-resolution RNA structures. The bond angles of the RNA backbone were then manually adjusted until the backbone fit into the cryo-EM density that was assigned to the RNA after the protein subunits were fit into the cryo-EM map. Last, standard bond angles and lengths of the backbone of the single-stranded RNA fragments were achieved with the use of the “Regularize Zone” tool in Coot. Because the RNA bases were not distinguishable in the 8.9 Å cryo-EM map, only the backbone of the single-stranded regions was modeled. For the pseudoatomic model of Teb1-Teb2-Teb3, RPA70C:RPA32N:RPA14 from the crystal structure of RPA (PDB ID 1L1O) (57) was fit into the cryo-EM maps by the colores program of Situs, using the exhaustive 6D search algorithm (76). The structure of the RPA trimer fit with high consistency into the cryo-EM map in the density assigned for Teb1C and the adjacent knob. For the final model, RPA70C was replaced by Teb1C, which fits in the same density, except for the C-terminal helix that is disordered in the Teb1C crystal structure but is apparently well ordered in the cryo-EM maps. RPA32N and RPA14 in the model are paralogs of Teb2 and Teb3, respectively. During the Situs fitting of RPA70C:RPA32N:RPA14 to the 9.4 Å cryo-EM map, a second location, corresponding to the tip of the density assigned to the p75-p45-p19 subcomplex, was identified with high confidence, which suggests that it was also an RPA homolog. For the pseudoatomic model of p75C-p45N-p19, RPA14 was manually replaced by p19 (PDB ID 5DFM), except for its C-terminal helix that is disordered in the p19 crystal structure. The crystal structure of the TPPI OB fold (PDB ID 2I46) (13) was placed in the cryo-EM density assigned to p50N and then fit using UCSF Chimera. The

cryo-EM maps and the modeled protein and RNA structures were visualized with UCSF Chimera and PyMOL (77).

Structure determination of p19, p45C, and TER PK

Full-length p19 (28) was fused via a triple alanine linker to the wild-type maltose binding protein (MBP) vector as described in (78), expressed in *Escherichia coli*, and purified by amylose affinity and size exclusion chromatography (SEC). To obtain diffracting native crystals, a 2:1 mixture of 12 mg/ml of MBP-p19 and the reservoir solution (0.1 M sodium acetate at pH 4.6 and 2.0 M ammonium sulfate) was set up in a 24-well hanging drop format. Full-length p45 (79) was fused to His-tagged MBP via a tobacco etch virus (TEV) protease cleavage site, expressed in *E. coli*, and copurified with cells expressing 6×His-tagged p19 using Ni-nitrilotriacetic acid (NTA) affinity and SEC. p45C (residues 162 to 373) was identified via limited chymotrypsin proteolysis of full-length p45 bound to p19. p45C was fused to His-tagged MBP via the TEV protease cleavage site, expressed in *E. coli*, and purified by Ni-NTA affinity and SEC. To obtain diffracting native crystals, a 1:1 mixture of 14 mg/ml of p45C and the reservoir solution [0.1 M Na-HEPES at pH 7.5, 5% (v/v) MPD, and 10% (w/v) polyethylene glycol 6000] was set up in a 24-well hanging drop format. Data were collected at 100 K at Advanced Photon Source–Northeastern Collaborative Access Team (APS-NECAT) beamline 24-ID-C on a DECTRIS PILATUS 6 M pixel detector. Data were indexed, integrated, and scaled using XDS/XSCALE (80). Structures were solved with the molecular replacement program PHASER (81), using initial models from SeMet data sets. Final models were iteratively built and refined in Coot (75) and PHENIX (82). In the crystal structure of p45C, β5 in WH2 is domain-swapped from a neighboring protein in the crystal lattice. This domain-swapped β5 was used to build a biological unit model of p45C (Fig. 5C). NMR assignments were obtained, and solution structure of the TER pseudoknot (nucleotides 68 to 100) was determined following established protocols for human and yeast TER pseudoknots (44, 45, 83). Sample conditions were ~1 mM RNA in 10 mM NaPO₄ at pH 6.3 and 50 mM KCl at 10°C. The structures were calculated with a total of 414 nuclear Overhauser effect distance, 171 dihedral angle, 82 H bond, and 79 residual dipolar coupling restraints using standard protocols in Xplor-NIH 2.9.8. Pairwise root mean square deviation for the 10 lowest-energy structures (out of 100) was 0.83 Å for all heavy atoms (table S4).

Mass spectrometry

Telomerase was purified from *Tetrahymena* TERT-FZZ and ZZ-F-p50 strains as described in (29), up to the final anti-FLAG resin wash step before elution. Telomerase was batch-washed on the resin three times by incubation in 1 ml of IGE-PAL-free wash buffer at 4°C, rotating end-over-end for 10 min, and was eluted twice by incubation at room temperature with 50 μl of 0.1% trifluoro-

acetic acid for 30 min each time. For enzymatic digestion of the samples, 70 μl of deoxycholic acid (0.1% w/v) in 1 M NH₄HCO₃ was added to the elution. Cysteines were alkylated by adding iodoacetamide to 4 mM and incubating at room temperature. After 30 min, excess dithiothreitol was added to quench residual iodoacetamide, and trypsin (300 ng) was added to initiate digestion. The solution was incubated at 37°C overnight, after which it was acidified to pH 2 with trifluoroacetic acid, to precipitate the deoxycholic acid. The deoxycholic acid was extracted from the aqueous layer with three 200-μl aliquots of water-saturated ethyl acetate (84). The digested peptides were dried and resuspended in 1% formic acid. The resulting tryptic peptides were measured by LC-MS/MS, using an EASY-nLC 1000 (Thermo Scientific, Waltham, MA) coupled to a Q-Exactive Orbitrap mass spectrometer (Thermo Scientific) and an EASY-Spray nano-electrospray ionization source. Peptides were injected onto a 75 μm by 15 cm, 3 μm, 100 Å PepMap C18 reversed-phase LC column and separated using a linear gradient from 5% solvent B (0.1% formic acid in acetonitrile) and 95% solvent A (0.1% formic acid in water) to 50% solvent B in 2 hours at a constant flow rate of 300 nl/min. Eluted peptides were analyzed with a top-ten data-dependent acquisition method and identified using Proteome Discoverer (version 1.4; Thermo Scientific) coupled to MASCOT (version 2.4.1; Matrix Science, London). Orbitrap MS resolving power was set to 70,000 at a mass/charge ratio (*m/z*) of 200 for MS1 and 17,500 at a *m/z* of 200 for MS2. Tryptic peptides with up to two missed cleavages were searched against a *T. thermophila* protein database (www.ciliate.org) supplemented with common protein contaminant and enzyme sequences (27,046 sequences), with dynamic modifications for carbamidomethyl (C), oxidation (M), deamidation (N, Q), and peptide N-terminal cyclization to eliminate NH₃ (Q, carbamidomethyl-cys) or H₂O (E) (C, Cys; M, Met; Q, Gln; E, Glu). Precursor- and product-ion mass tolerances were set to 10 parts per million (or less) and 0.01 daltons, respectively.

Telomerase reconstitution and activity assays

Telomerase subcomplexes were assembled separately and then combined. The RNP catalytic core was assembled by TERT expression in RRL in the presence of purified bacterially expressed p65 and in vitro transcribed TER. Additional RRL synthesis reactions generated p50N30, Teb1, or coexpressed TEB complex subunits. One subunit was tagged with 3×FLAG (p50N30-F for TEB subunit function studies or TERT-F for TERT TEN domain mutational analysis). Subcomplexes were combined, bound to FLAG antibody resin, and assayed by primer extension as described previously (29), using radiolabeled deoxyguanosine triphosphate (dGTP) and primer (GTTGGG)₃ at a final concentration of 200 nM. Elongation time was 10 min unless otherwise indicated. Products were resolved by denaturing gel electrophoresis, including an end-labeled oligonucleotide added before product precipitation as a recovery control.

Preparation of DNA-LNA-bound telomerase linked by streptavidin and negative-stain EM

Three-repeat biotinylated LNA/DNA primer was purchased from Exiqon as biotin-5'-GTTGGGG-TTGGGGT_LT₁G_LG_LG. Telomerase was purified as described (29) from TERT-FZZ strain up to the TEV protease cleavage step, during which 5 μ M three-repeat primer and 50 μ M dGTP were added to the immunoglobulin G resin. Immediately before the 10-min wash steps of the telomerase-bound flag resin, the resin was split, and one-half was incubated in 50 μ l of wash buffer, whereas the other was incubated in 50 μ l of wash buffer with 5 μ M streptavidin, rotating end-over-end at 4°C for 30 min. Three wash steps were then performed as usual, after which the two resins were recombined and batch-eluted with 3 $\times$ FLAG peptide, according to the original protocol. Negative-stain EM samples were prepared, imaged, and processed as previously described (29).

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SUPPLEMENTARY MATERIALS

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Figs. S1 to S10
Tables S1 to S4
References (85–88)

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RESEARCH ARTICLE

BATTERIES

Cycling Li-O₂ batteries via LiOH formation and decompositionTao Liu,¹ Michal Leskes,^{1†} Wanjiang Yu,^{1,2‡} Amy J. Moore,¹ Lina Zhou,¹ Paul M. Bayley,¹ Gunwoo Kim,^{1,2} Clare P. Grey^{1*}

The rechargeable aprotic lithium-air (Li-O₂) battery is a promising potential technology for next-generation energy storage, but its practical realization still faces many challenges. In contrast to the standard Li-O₂ cells, which cycle via the formation of Li₂O₂, we used a reduced graphene oxide electrode, the additive LiI, and the solvent dimethoxyethane to reversibly form and remove crystalline LiOH with particle sizes larger than 15 micrometers during discharge and charge. This leads to high specific capacities, excellent energy efficiency (93.2%) with a voltage gap of only 0.2 volt, and impressive rechargeability. The cells tolerate high concentrations of water, water being the dominant proton source for the LiOH; together with LiI, it has a decisive impact on the chemical nature of the discharge product and on battery performance.

Rechargeable nonaqueous lithium-air (Li-O₂) batteries have attracted considerable interest over the past decade, because of their much higher theoretical specific energy than conventional Li ion batteries (1–3). A typical Li-O₂ cell is composed of a Li metal negative electrode, a nonaqueous Li⁺ electrolyte, and a porous positive electrode. During discharge, O₂ is reduced and combines with Li⁺ at the positive electrode, forming insoluble discharge products (typically Li₂O₂) that fill up the porous electrode (4–6). The porous electrode is not the active material but rather a conductive stable framework that hosts the reaction products, lighter electrode materials providing higher specific energies. During charge, the previously formed discharge products must be thoroughly removed to prevent the cell from suffocating after a few discharge/charge cycles, the electrode pores becoming rapidly clogged with discharge products and products from unwanted side reactions (7–13).

Several fundamental challenges still limit the practical realization of Li-O₂ batteries (1–3). The first one concerns the reversible capacity (and thus energy density). This is determined by the pore volume of the porous electrode, which limits both the total quantity of the discharge products and how large the discharge product crystals can grow. The ultimate capacity—currently far from being reached—is, in theory, achieved in the extreme case in which large single crystals of the discharge product grow to occupy the full geometric volume of the positive electrode. The commonly used mesoporous Super P (SP)/Ketjen carbon electrodes have relatively small pore sizes and volumes, with their crystalline discharge products typically less

than 2 μm in size (4, 5, 14); this limits the capacity to <5000 milli-ampere-hour per gram of carbon (mA·h/g) (typically <1.5 mAh based on 1 mg of carbon and binder) (5, 7–10, 12, 13). In addition, uses of smaller pores tend to lead to pore clogging, hindering the diffusion of O₂ and Li⁺ and causing high overpotentials during cycling. Second, severe side reactions can occur on cycling, involving the electrode materials, electrolyte, and intermediate as well as final discharge products (7–13). Major causes of these decomposition reactions include the superoxide ion that forms as an intermediate on reduction of oxygen, which readily attacks most electrolytes (7–9, 15), and the large overpotential on charge, often required to remove the insulating

discharge products, which results in the oxidation of some of the cell components such as the host electrode (10–13). Previous studies (10–13) suggest that 3.5 V (versus Li/Li⁺) represents the maximum voltage that carbon-based electrodes can tolerate without significant side reactions. Third, the large hysteresis seen between charge and discharge (up to 2 V) (4–13), results in extremely low energy efficiencies, limiting the use of this battery in practical applications. Finally, the cells are very sensitive to moisture and carbon dioxide (16–19): The more stable LiOH and Li carbonate phases are formed, which gradually accumulate in the cell, resulting in battery failure. Moisture and CO₂ also have deleterious effects on the Li-metal anode (1–3).

A number of strategies have been proposed to reduce the voltage hysteresis, involving the use of electrocatalysts (20–27), porous electrode structures (28–30), and redox mediators (31–35). Soluble redox mediators, such as tetrathiafulvalene (TTF) (32) and LiI (34), have been used to reduce the overpotential of the charge process, resulting in the overall voltage hysteresis dropping to around 0.5 V (34). Their operation relies on the electrochemical oxidation of the mediator, which itself then chemically decomposes the Li₂O₂. The charge voltage is thus tuned close to the redox potential of the mediator. For discharge, the ethyl viologen redox couple (31) has also been used to reduce O₂ in the liquid electrolyte rather than on the solid electrode surface, again to help prevent rapid blocking of the solid electrode surface by Li₂O₂. We used the redox mediator LiI and report a Li-O₂ battery with an extremely high efficiency, large capacity, and a very low overpotential. This battery cycles via LiOH formation, not Li₂O₂, and is able to tolerate large quantities of water. This current work directly addresses a number of critical issues associated with this battery technology.

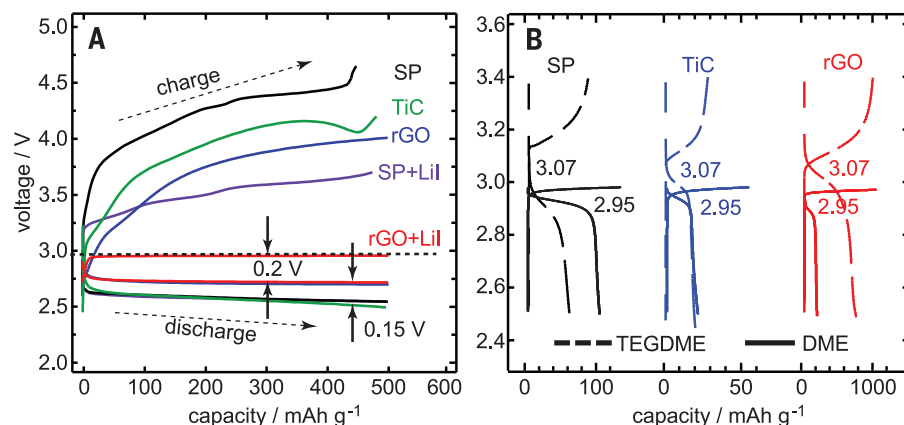


Fig. 1. Electrochemical profiles of cells with different electrode/electrolyte combinations. (A) Discharge-charge curves for Li-O₂ cells using mesoporous SP and TiC, and macroporous rGO electrodes, with capacities limited to 500 mA·h/g (based on the mass of carbon or TiC) and a 0.25 M LiTFSI/DME electrolyte. For SP and rGO electrodes, 0.05 M LiI was added to the LiTFSI/DME electrolyte in a second set of electrodes (purple and red curves). All cells in (A) were cycled at 0.02 mA/cm². The horizontal dashed line represents the position (2.96 V) of the thermodynamic voltage of a Li-O₂ cell. (B) Galvanostatic charge-discharge curves of cells containing 0.05 M LiI and 0.25 M LiTFSI, cycled under an Ar atmosphere with different electrode/electrolyte solvent combinations with a current of 0.2 mA/cm². The crossing points (with appropriate voltages labeled) of the charge/discharge curves indicate the positions of the redox potential of I[•]/I₃[•] in the specific electrode/electrolyte system. A direct comparison of capacities between LiI in Ar and Li-O₂ cells is given in fig. S3.

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A Li-O₂ battery was prepared by using a Li metal anode, a 0.25 M lithium bis(trifluoromethyl) sulfonimide/dimethoxyethane (LiTFSI/DME) electrolyte with 0.05 M LiI additive, and a variety of different electrode structures (fig. S1). Hierarchically macroporous reduced graphene oxide (rGO) electrodes (binder-free) are used because they are light, conductive, and have a large pore volume that can potentially lead to large capacities. Mesoporous SP carbon and mesoporous titanium carbide (TiC) (36) electrodes were studied for comparison. Cyclic voltammetry (CV) measurements confirmed that rGO, SP, and TiC electrodes all exhibit good electrochemical stability within a voltage window of 2.4 to 3.5 V in a LiTFSI/DME electrolyte and can be used to reversibly cycle LiI ($\text{I}_3^- + 2\text{e}^- \leftrightarrow 3\text{I}^-$) (37) (fig. S2).

In the absence of LiI, cells using either mesoporous TiC or macroporous rGO showed much smaller overpotentials during charge, in comparison to that obtained with the SP electrode (Fig. 1A). These decreases in overpotential are tentatively ascribed to the higher electrocatalytic activity of TiC (38) and the faster diffusion of Li⁺ and solvated O₂ within the micrometer-sized pores of the rGO electrodes (fig. S1). The addition of LiI to the SP electrode led to a noticeable drop in the overpotential over that seen with SP only, suggesting that the polarization during charge is largely caused by the insulating nature of the discharge products. The charge voltage profile is not, however, flat, but gradually increases as the charge proceeds to above 3.5 V. In contrast, when LiI is

used with hierarchically macroporous rGO electrodes, a remarkably flat process is observed at 2.95 V, representing a further reduction in overpotential by ~0.5 V over that seen for SP. This reduction is ascribed, at least in part, to the interconnecting macroporous network of rGO, which allows for much more efficient mediator diffusion than in the mesoporous SP electrode, even when the macropores are filled with insoluble discharged products.

The observation that the LiI/DME Li-O₂ cell charges at 2.95 V is of note, because it is slightly below the thermodynamic voltage of 2.96 V of the Li-O₂ reaction. During charge, the redox mediator is thought to be first electrochemically oxidized on the electrode (32); this oxidized form then helps to chemically decompose the discharge product. The charge voltage then reflects the redox potential (versus Li/Li⁺) of the I[•]/I₃^{•-} redox mediator in the electrode/electrolyte system rather than the redox potential associated with the oxidation of the solid discharge product. A low redox potential of a mediator is important for the long-term stability of the Li-O₂ cell.

To investigate factors affecting the redox potential, LiI was cycled galvanostatically in an Ar atmosphere with different electrode/electrolyte combinations (Fig. 1B). The electrolyte solvent has a larger effect on the redox potential of the I[•]/I₃^{•-} couple than the electrode material, with the DME electrolyte consistently exhibiting lower charge voltages than TEGDME (tetraethylene glycol dimethyl ether) for all three electrodes. In addition, the voltage gaps between the charge and discharge

plateaus are smaller for DME than for TEGDME electrolytes, which is consistent with the smaller voltage separations seen between the redox peaks in their respective CV curves (fig. S2). The discharge capacity is always smaller than the previous charge capacity for all cells (Fig. 1B), indicating that some mediators, after being oxidized, have diffused into the bulk electrolyte. This observation is more prominent with DME, suggesting faster mediator diffusion in DME than in TEGDME.

The discharge overpotential for rGO-based Li-O₂ cells also decreases by 0.15 V (marked by arrows in Fig. 1A), from 2.6 (SP/TiC) to 2.75 V (rGO), regardless of the use of LiI. Overall, the voltage gap becomes only 0.2 V (indicated by arrows), representing an ultrahigh energy efficiency of 93.2%.

X-ray diffraction (XRD) patterns (Fig. 2A) for the rGO electrodes cycled with LiI show that LiOH is the only observed crystalline discharge product; LiOH is then removed after a full charge. This is confirmed in the solid-state nuclear magnetic resonance (ssNMR) measurements (Fig. 2B), where a single resonance due to LiOH is observed at -1.5 ppm and at 1.0 ppm in the ¹H and ⁷Li magic angle spinning ssNMR spectra (13, 39), respectively (further corroborated by the ⁷Li static NMR spectrum in fig. S4). After charge, the ¹H and ⁷Li LiOH resonances are no longer visible. We emphasize that without added LiI, the predominant discharge product for rGO electrodes is Li₂O₂ (fig. S5), the chemistry radically changing when 0.05 M LiI is added to the DME electrolyte.

Figure 2, C and D, shows optical and scanning electron microscopy (SEM) images of electrodes during the first cycle. After discharge, the electrode surface is completely covered by LiOH agglomerates, tens of microns in size, and the color of the electrode has changed from black to white. When the interior of the electrode was investigated, many crystalline “flowerlike” agglomerated LiOH particles were observed within the graphene macropores. Although these particles are more than 15 μm in diameter (fig. S6), much bigger than the Li₂O₂ toroids (fig. S5), they are in fact formed from thin-sheet primary building blocks, resulting in a more open (porous) structure. The large LiOH agglomerates efficiently fill up the pore volume available in the hierarchical macroporous electrode, leading to much larger capacities (fig. S6). When TEGDME was used as the electrolyte solvent, the discharge product, although still LiOH, now forms a thin film on the rGO electrode surface (fig. S7). After charge in DME, the hierarchically macroporous structure reappeared and the electrode turned black again (Fig. 2C). Higher-magnification SEM images revealed very small traces of residual LiOH on the electrode surface (fig. S8). We found that the reversible formation and removal of LiOH with the LiI mediator are not restricted to rGO electrodes, because mesoporous SP electrodes show similar results (fig. S9) but with larger overpotentials and lower capacities.

Kang and co-workers (34) previously reported a highly rechargeable Li-O₂ cell using carbon nanotubes and the mediator LiI (0.05 M) in a TEGDME-based electrolyte, ascribing the electrochemistry to the formation and decomposition of Li₂O₂.

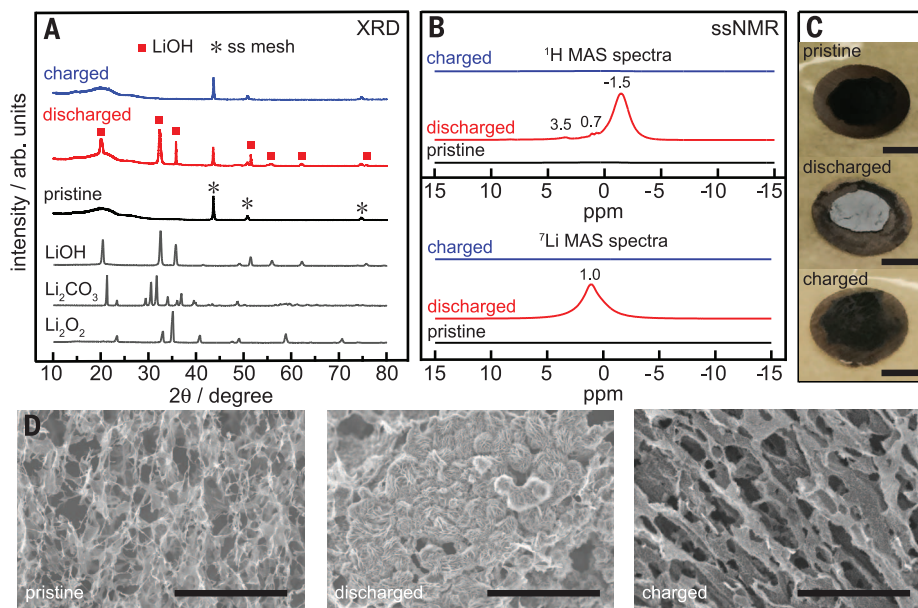


Fig. 2. Characterization of pristine, discharged and charged electrodes. XRD patterns (A) and ¹H and ⁷Li ssNMR spectra (B) comparing a pristine rGO electrode to electrodes at the end of discharge and charge in a 0.05 M LiI/0.25 M LiTFSI/DME electrolyte (the electrochemistry of the cells is in fig. S10). The spectra are scaled according to the mass of the pristine electrode and the number of scans. Asterisks in (A) represent diffraction peaks from the stainless steel mesh. ¹H resonances of proton-containing functional groups in the pristine rGO electrode are not visible in the ¹H ssNMR spectrum in (B), because they are very weak in comparison to the LiOH signal. The weaker signals at 3.5 and 0.7 ppm are due to DME and grease/background impurity signals, respectively. Optical (C) and SEM (D) images of pristine, fully discharged and charged rGO electrodes obtained with a 0.05 M LiI/0.25 M LiTFSI/DME electrolyte in the first cycle. The scale bars are all 5 mm and 20 μm in the optical and SEM images, respectively.

Sun *et al.* (35) recently pointed out, however, that LiOH, rather than Li_2O_2 , was the dominant discharge product when 0.05 M LiI mediator was added to the TEGDME electrolyte; LiOH was still present in the SP electrode used in their study after charge, and they suggested that LiOH could not be decomposed by the mediator. In our work, we have seen clear evidence that the discharge product is overwhelmingly LiOH and that it can be removed at low potentials of around 3 V.

In a redox-mediated Li- O_2 system, the effective removal of the insulating discharge products is affected by a few factors: (i) availability of bare electrode surfaces to oxidize the mediator during charge, (ii) whether the discharge product is uniformly distributed throughout the electrode, and (iii) efficient diffusion of the oxidized mediator from electrode surfaces (that supply and remove electrons) to the discharge product. TEGDME, being a more viscous solvent than DME, will lead to more sluggish I_3^- and O_2 diffusion. When it is used with mesoporous (rather than macroporous) electrodes, the discharge product tends to concentrate on the electrode surface facing the gaseous O_2 reservoir, with its concentration dropping noticeably in the electrode interior [the reaction zone problem for Li-air batteries (40)]. The much more soluble LiI, however, is likely to be uniformly oxidized across the whole thickness of the electrode during charge. Consequently, for equal capacities for discharge and charge, the oxidized mediator (I_3^-) formed during charge may remain in excess in the electrode interior regions, where the discharge product is scarce; similarly, discharge product may be left unreacted at regions close to the O_2 /electrolyte interface, where the discharge product is abundant. The remaining mediator in the oxidized form (I_3^-) will then be reduced during the next discharge, resulting in a voltage plateau at its redox potential in addition to that due to oxygen reduction. This unbalanced distribution of the mediator LiI and the discharge product LiOH across the thickness of the mesoporous electrode may be a cause of the unreacted LiOH and iodine-dominated electrochemistry observed in the work by Sun and co-workers (33). Furthermore, the thin-film morphology of the discharge product formed in the TEGDME-based electrolyte [fig. S7 and (35)] effectively passivates the electrode surface. As a result, triiodide anions may first form on bare electrode surfaces that are distant from the discharge products. They then need to diffuse to the passivated regions to remove LiOH, reducing the efficiency of LiOH removal and providing another explanation for the observed residual LiOH. We used a macroporous rGO electrode and DME, which provides faster mediator and O_2 diffusion, and higher LiO_2 solubility, leading to a more uniform Li- O_2 reaction during discharge and larger reversible capacities.

Figure 3 shows the electrochemical performance of the Li- O_2 battery. When limiting the specific capacity to 1000, 5000, and 8000 mA-h/g_c, the cells show no capacity fade, with little increase in voltage polarization after 2000, 300, and 100 cycles, respectively (Fig. 3, A to C). Higher capacities >20,000 mA-h/g_c have also been demonstrated

(figs. S5 and S11). When cycled at 1 A/g_c (Fig. 3, A and C), the voltage gap is only ~0.2 V; at higher rates, the gaps widen (Fig. 3D), increasing to 0.7 V at 8 A/g_c. Furthermore, at this higher rate, the cell is polarized each cycle (fig. S11), and after 40 cycles the electrode surface is covered by a large number of particles (with morphologies unlike those of LiOH observed at lower currents), which do not seem to be readily removed during charge at these voltages. At these higher overpotentials, more substantial parasitic reactions probably occur, rapidly polarizing the cell by increasing its resistance and impeding the electron transfer across the electrode/electrolyte interface. A narrower operating electrochemical window within 2.96 ± 0.5 V is key for the prolonged stability of the rGO electrodes.

The sensitivity of the cell to water was explored by either deliberately adding ~45,000 parts per million (ppm) of H_2O (37 mg per 783 mg of DME) to the electrolyte or cycling cells under a humid O_2 atmosphere. In both cases, no appreciable change in the electrochemical profile was observed (fig. S12), compared to that using a nominally dry electrolyte. Furthermore, the added water was found to promote the growth of even larger LiOH crystals >30 μm (fig. S13).

Although a certain level of scattering in the total capacity is observed, probably due to variations in the electrode structure, the cell capacity is typically within 25,000 to 40,000 mA-h/g_c (i.e., 2.5 to 4.0 mA-h) range for an rGO electrode of 0.1 mg and 200 μm thick. After discharge, the weight of an electrode removed from the stainless steel mesh was about 1.5 mg (2.7 V, 3.2 mA-h), giving a specific energy of 5760 W-h/kg (see section 13 of the supplementary materials).

The mechanism of O_2 reduction in aprotic Li- O_2 batteries has been extensively discussed. Many authors (5, 41, 42) have shown that the ability of an electrolyte to solvate O_2^- (characterized by the Guttmann donor number, DN) is important in governing the discharge reaction mechanism. Higher LiO_2 solubility favors a solution precipitation mechanism leading to large toroidal Li_2O_2 crystals and thus higher discharge capacity; lower LiO_2 solubility tends to drive a surface mechanism where Li_2O_2 forms thin films on the electrode surface and a lower capacity. Because of its intermediate DN, solution precipitation and surface reduction mechanisms can occur simultaneously in DME (41).

With added LiI, although LiOH rather than Li_2O_2 is the prevailing discharge product, many parallel phenomena are observed: The similar discharge voltages (2.75 V, Fig. 1A) observed with and without the added LiI suggest that the first step on discharge is an electrochemical reaction, where O_2 is reduced on the electrode surface to form LiO_2 . It is unlikely that O_2 is reduced to O_2^{2-} via two-electron electrochemical steps or even dissociatively reduced to O^{2-} (or LiOH) via four-electron electrochemical steps. Subsequent conversion of LiO_2 to LiOH is proposed to be a chemical process that occurs via a solution mechanism. Strong support for a solution mechanism comes from the observation that LiOH grows on both the electrode and the insulating glass fiber separators (fig. S6), the latter not being electrically connected to the current collector. This process must involve the iodide redox mediator, because in its absence Li_2O_2 is formed, even in cells with high moisture contents.

A key question is the origin of the H^+ in the formed LiOH, potential sources being the DME

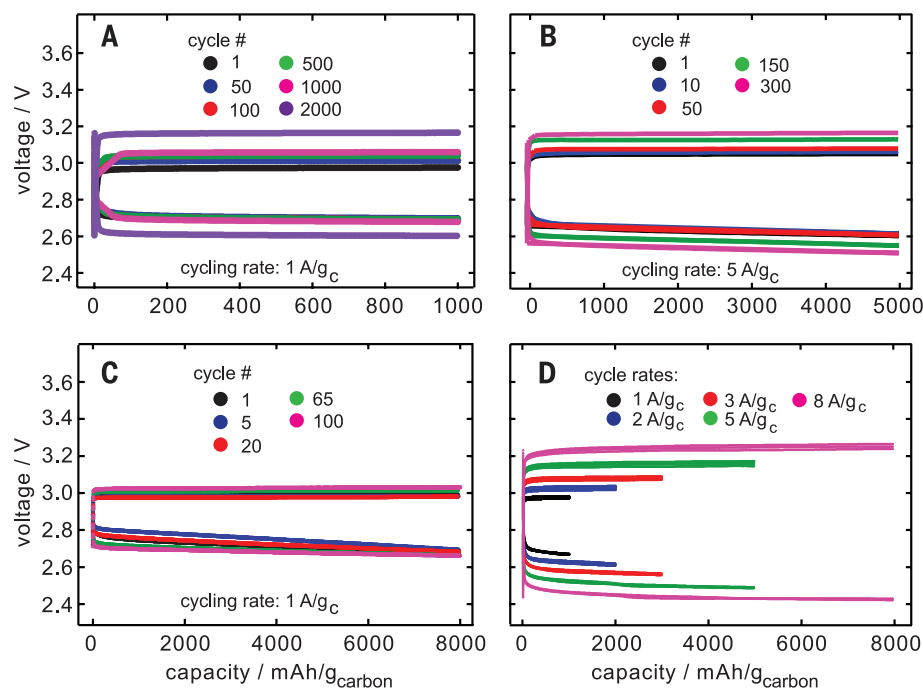


Fig. 3. Electrochemical performance of the Li- O_2 battery. Discharge/charge curves for Li- O_2 batteries using rGO electrodes and a 0.05 M LiI/0.25 M LiTFSI/DME electrolyte with capacity limits of 1000 mA-h/g_c (A), 5000 mA-h/g_c (B), and 8000 mA-h/g_c (C), as a function of rate (D); three cycles were performed for each rate in (D). The cell cycle rate is based on the mass of rGO; i.e., 5 A/g_c is equivalent to 0.1 mA/cm².

electrolyte, surface functional groups of rGO, and moisture in the cell (fig. S14). To investigate this, NMR measurements were conducted on two sets of discharged electrodes from cells that were prepared using either deuterated DME or deuterated water (figs. S15 to S18). When deuterated DME was used, only a very small quantity of LiOD was detected in the ^2H NMR spectra (figs. S15, S16, and S18), the dominant product being LiOH. In contrast, when D_2O was added to the protonated DME electrolyte, a significant amount of LiOD was observed (fig. S17), LiOH only being a minor signal in the corresponding ^1H NMR spectrum. In summary, these experiments clearly demonstrate that (i) although DME can be a potential H^+ source for LiOH, it is by no means the dominant one; (ii) the added water preferentially supplies H^+ to form LiOH, substantially minimizing DME decomposition (figs. S17 and S18); and (iii) even our nominally dry cells contain sufficient water to promote LiOH formation. This latter statement is consistent with the formation of large toroidal Li_2O_2 particles in the absence of LiI (fig. S5); earlier work shows that this requires water levels of >500 ppm (42).

^1H NMR spectroscopy was used to quantify the number of moles of LiOH formed on discharge (with added LiI mediator). The (molar) ratio of electrons consumed on discharge to LiOH formed was close to 1:1 within the errors of the measurements (fig. S19), supporting the proposal that LiOH is formed in stoichiometric quantities (i.e., is not a minor side product).

During charge, an iodine-mediated LiOH decomposition reaction is observed at ~ 3 V, a clear distinction of our work from that of others (34, 35). Given that this charge voltage overlaps with that for I^-/I_3^- itself (measured with Ar gas in Fig. 1B), the first step should involve the direct electrochemical oxidation of I^- to I_3^- . We suggest that the next step involves the chemical oxidation of LiOH by I_3^- to form O_2 and H_2O . To confirm this hypothesis, a pre-discharged Li- O_2 cell (with LiI) was charged in a pure Ar atmosphere, and the gas atmosphere in the cell after charge was monitored by mass spectrometry. A clear O_2 signal (fig. S21) was detected, consistent with the proposal that LiOH decomposition occurs via an O_2 evolution reaction. The discharge and charge reactions are schematically represented in Fig. 4. We

stress, however, that the equilibria that occur in the presence of oxygen, water, and iodine are complex, often involving a series of polyanions (including IO^- and its protonated form); further mechanistic studies are required to understand the role of these complex equilibria in the redox processes.

By using an rGO electrode and the redox mediator LiI, in a DME-based electrolyte, we have demonstrated a highly efficient, rechargeable Li- O_2 battery with extremely large capacities. Its operation involves the reversible formation and removal of LiOH crystals. The role of the additive, LiI, is threefold. First, it operates as a redox mediator whose redox potential can be tuned by using different electrolyte solvents and electrode structures; this redox potential guides the charge voltage and thus affects the cycling stability of the cell. Second, LiI, together with another additive H_2O , affects the chemical nature and physical morphology of the discharge products, inducing the growth of large LiOH crystals that efficiently take up the pore volume of macroporous rGO electrodes; this is the origin of the observed large capacity. Finally, it enables a chemical pathway to remove LiOH at low overpotentials. Consequently, the cell becomes insensitive to relatively high levels of water contamination. The hierarchically macroporous rGO electrode is also an important factor for the high efficiency and capacity. Not only does the rGO framework provide efficient diffusion pathways for all redox active species in the electrolyte and hence, a reduced cell polarization and flatter electrochemical profile, it also permits the growth of LiOH crystals of tens of micrometers in size, resulting in a capacity that is much closer to the theoretical value of Li- O_2 batteries. These desirable features were not observed for Li- O_2 cells with mesoporous SP electrodes, even when the same electrolyte was used. The combination of electrolyte additives, the porous electrode structure, and the electrolyte solvent, synergistically, not only determines the chemical nature of the discharge product but also governs the physical size and morphology of it, playing a decisive factor in the capacity and rechargeability of the resulting Li- O_2 battery. In a broader sense, this work can inspire ways to remove other stable, detrimental chemicals, such as Li_2CO_3 , which is relevant to cycling Li-air batteries in real practical conditions.

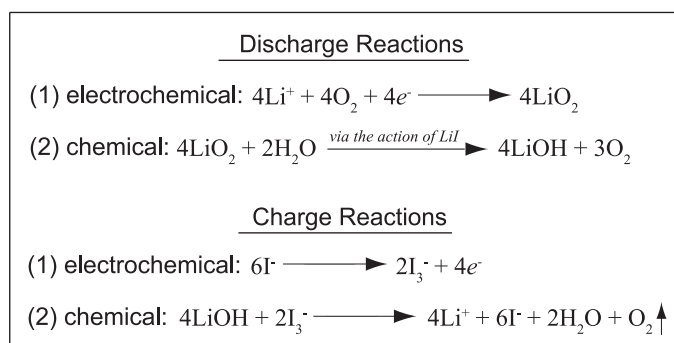


Fig. 4. Schematic mechanisms for the formation and removal of LiOH in iodide redox-mediated Li- O_2 cells in the presence of water. The electron/LiOH molar ratios during discharge and charge are both equal to 1.

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SUPPLEMENTARY MATERIALS

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REPORTS

GEOMORPHOLOGY

Geophysical imaging reveals topographic stress control of bedrock weathering

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Bedrock fracture systems facilitate weathering, allowing fresh mineral surfaces to interact with corrosive waters and biota from Earth's surface, while simultaneously promoting drainage of chemically equilibrated fluids. We show that topographic perturbations to regional stress fields explain bedrock fracture distributions, as revealed by seismic velocity and electrical resistivity surveys from three landscapes. The base of the fracture-rich zone mirrors surface topography where the ratio of horizontal compressive tectonic stresses to near-surface gravitational stresses is relatively large, and it parallels the surface topography where the ratio is relatively small. Three-dimensional stress calculations predict these results, suggesting that tectonic stresses interact with topography to influence bedrock disaggregation, groundwater flow, chemical weathering, and the depth of the "critical zone" in which many biogeochemical processes occur.

Weathered bedrock and soil are part of the life-sustaining layer at Earth's surface commonly referred to as the "critical zone." Its thickness depends on the competition between erosion, which removes weathered material from the land surface, and weathering, which breaks down rock mechanically and chemically and thus deepens the interface between weathered and fresh bedrock (1–3). Erosion at the surface can be studied both directly by observation (4) and indirectly using isotopic tracers (5). In contrast, weathering at depth is generally obscured by overlying rock and soil, making it more difficult to study. In this Report, we combine results from landscape-scale geophysical imaging and stress-field modeling to evaluate hypotheses about the relationship of subsurface weathering to surface topography.

One hypothesis is that weathering at depth is regulated by the hydraulic conductivity and porosity of bedrock and the incision rate of river channels into bedrock, which together determine

how rapidly groundwater flowing toward the river channels can drain bedrock pores of chemically equilibrated water (1). Another hypothesis, based on reactive transport modeling, predicts that the propagation of the weathered zone depends on the balance between mineral reaction kinetics and groundwater residence times (2, 6). These mechanisms are not mutually exclusive, and both emphasize the importance of fluid flow through bedrock. This implies that the development of fracture systems, which provide preferred fluid-flow paths in bedrock (7, 8), should help regulate the downward propagation of weathering.

Bedrock fractures may be inherited from past tectonic or thermal events, but the common observation that fracture abundance declines with depth (9) suggests that near-surface processes are capable of generating new fractures or reactivating existing ones. Examples of potential near-surface fracturing mechanisms include wedging due to growth of ice crystals (10), salt crystals (11), or roots (12); volume expansion due to weathering reactions of certain minerals (13); and stress perturbations associated with surface topography (14). Some of these mechanisms require specific conditions that only occur in certain geographic regions, rock types, or parts of the subsurface. For example, ice weathering requires repeated freeze-thaw cycles and is therefore limited to depths of a few meters, and volume expansion due to mineral weathering is most effective in rocks containing abundant biotite or hornblende. In contrast, surface topography always perturbs the bedrock stress field. Here we focus on this potentially widespread control on bedrock fractures.

Bedrock stress fields can be evaluated by considering the effects of local topography on grav-

itational stresses and regional tectonic stresses. Theoretical calculations have shown that the presence of topographic features such as ridges and valleys can cause vertical and lateral variations in the shallow subsurface stress field (15) and that these stress perturbations can be large enough to alter bedrock fracture patterns (14, 16). Some observational evidence suggests that topographic stresses influence near-surface fracture distributions (9, 17, 18) and groundwater flow (19–21), but it is unknown whether topographic stresses systematically affect the spatial distribution of bedrock weathering. In this study, we used geophysical surveys of seismic velocity and electrical resistivity to image the thickness of the weathered zone in three landscapes with similar topography but different tectonic stress conditions, and we tested whether the geometry of the weathered zone in each landscape matches the modeled topographic stress field.

A calculation of the stresses beneath an idealized topographic profile in the presence of regional tectonic stress illustrates how topographic stress might influence the geometry of the weathered zone (Fig. 1). We calculated the total stress field as the sum of the ambient stress due to gravity and tectonics and the stress perturbation due to topography (22). From the stress field, we calculated two scalar quantities that act as proxies for two mechanisms that could influence the abundance of fractures. As a proxy for shear fracturing or shear sliding on existing fractures, we calculated the failure potential (Φ) (23), defined as $(\sigma_{mc} - \sigma_{lc})/(\sigma_{mc} + \sigma_{lc})$, where σ indicates a stress, and the subscripts denote the most compressive (mc) and least compressive (lc) principal stresses, with compression being positive. As a proxy for opening-mode displacement on fractures, we used the magnitude of σ_{lc} . Because we do not have prior knowledge of the orientations or abundances of existing fractures in a given landscape, we used both quantities to represent the propensity for generating open fractures. A larger Φ indicates that new shear fractures are more likely to form and that existing fractures oriented obliquely to the σ_{mc} and σ_{lc} directions are more likely to dilate as a result of sliding on the rough fracture surfaces (24). A smaller σ_{lc} indicates that fractures oriented nearly perpendicular to the σ_{lc} direction will be more likely to open (20, 25). Given that open fractures permit water to flow more quickly through bedrock and enhance the rate of chemical weathering, we expect that zones of larger Φ or smaller σ_{lc} correspond to zones of more weathered bedrock.

In a scenario with a horizontal land surface and a stress field determined by both gravity and a low, uniform ambient horizontal compression, Φ declines and σ_{lc} increases with depth beneath the land surface (Fig. 1, A and D). If topography is added and the ambient horizontal compression remains low, contours of Φ and σ_{lc} generally parallel the surface (Fig. 1, B and E). This surface-parallel pattern occurs because σ_{lc} is determined primarily by the overburden in this scenario, and therefore it increases with

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depth similarly beneath ridges and valleys (fig. S1A). However, if the ambient horizontal stress becomes strongly compressive—so that σ_{mc} at shallow depths parallels the topographic surface, and σ_{lc} becomes small relative to σ_{mc} (fig. S1, E and F)—then contours of Φ and σ_{lc} resemble mirror images of the land surface, plunging below ridges and rising beneath valleys (Fig. 1, C and F). This mirror-image pattern occurs because σ_{lc} becomes much smaller beneath ridges than beneath valleys, whereas the fractional decrease in σ_{mc} is relatively minor. In all three scenarios, spatial variations in σ_{lc} are the dominant cause of the spatial variations in Φ (Fig. 1, A to F, and fig. S1).

Where contours of Φ and σ_{lc} are generally parallel to the land surface, we expect to find a zone of abundant open fractures and weathered bedrock with a roughly uniform thickness, underlain by less weathered bedrock with fewer open fractures (Fig. 1, G and H). Where contours of Φ and σ_{lc} mirror the land surface, we expect to find thick weathered zones beneath ridges and thin zones beneath valleys (Fig. 1I). This conceptual model assumes that the geometry of the weathered zone reflects the present-day stress field. In an eroding landscape, a parcel of rock experiences a time-varying stress field as the eroding surface draws nearer. However, given that Φ increases and σ_{lc} decreases toward the surface (provided that the ambient horizontal tectonic stresses are compressive) (Fig. 1, A to F), the bottom boundary of the weathered zone is likely to be demarcated by the contour of the

lowest Φ or highest σ_{lc} at which fractures open enough to accelerate chemical weathering. After crossing this boundary, rocks will only experience higher Φ and lower σ_{lc} and should become even more weathered.

A dimensionless ratio of tectonic stresses to gravitational stresses that accounts for topographic perturbations captures the difference between the surface-parallel scenario (Fig. 1, B, E, and H) and the surface-mirroring scenario (Fig. 1, C, F, and I). This ratio is defined as

$$\sigma^* = \frac{\sigma_t b/a}{\rho g b} = \frac{\sigma_t}{\rho g a} \quad (1)$$

where σ_t is the magnitude of the horizontal tectonic stress perpendicular to the ridges and valleys, which we refer to as the tectonic compression; b/a is the characteristic slope of a topographic feature with height b and horizontal length a ; ρ is rock density; and g is gravitational acceleration. Our model predicts that a landscape with $\sigma^* < \sim 1$, corresponding to low tectonic compression or widely spaced ridges and valleys, should have a zone of large Φ , small σ_{lc} , and weathered rock that parallels the surface topography (Fig. 1, B, E, and H). Conversely, a landscape with $\sigma^* > \sim 1$, corresponding to high tectonic compression or closely spaced ridges and valleys, should have a zone of large Φ , small σ_{lc} , and weathered rock that thickens under ridges and thins under valleys (Fig. 1, C, F, and I). The surface-mirroring pattern is most pronounced for landscapes with $b/a > 0.05$.

To test our conceptual model, we investigated three field sites in the United States with similar topography but different ambient tectonic stress regimes. Gordon Gulch (Fig. 2B), in the Boulder Creek Critical Zone Observatory, Colorado (3), is underlain by Precambrian gneiss and has weak tectonic compression ($\sigma_t = 1$ MPa; $\sigma^* = 0.2$). Calhoun Critical Zone Observatory, South Carolina (Fig. 2C) (26), is underlain by Neoproterozoic or Cambrian granite gneiss and has stronger tectonic compression ($\sigma_t = 4.7$ MPa; $\sigma^* = 1.2$). Pond Branch, Maryland (Fig. 2D) (27), is underlain by Precambrian schist and has the strongest tectonic compression of the three sites ($\sigma_t = 8.4$ MPa; $\sigma^* = 3.2$). At each site, we selected a valley with a cross-sectional relief of 20 to 40 m and used a three-dimensional stress model to calculate the topographic perturbation to the ambient stress field in the vicinity of the valley (22). Our model calculations include ~ 1 to 15 km² of the topography surrounding our field sites (Fig. 2). Ambient stress magnitudes, directions, and depth gradients were constrained from compilations of regional measurements (fig. S2) (22).

Transects of Φ and σ_{lc} across the valleys (Fig. 3, A to F) show patterns consistent with the idealized calculations shown in Fig. 1, A to F. A zone of high Φ and low σ_{lc} , with roughly uniform depth, parallels the land surface at Gordon Gulch ($\sigma^* = 0.2$) (Fig. 3, A and D), whereas the zones of high Φ and low σ_{lc} at Calhoun ($\sigma^* = 1.2$) and Pond Branch ($\sigma^* = 3.2$) are deeper beneath ridges and shallower beneath valleys (Fig. 3, B, C, E, and F). Sensitivity tests accounting for uncertainties in

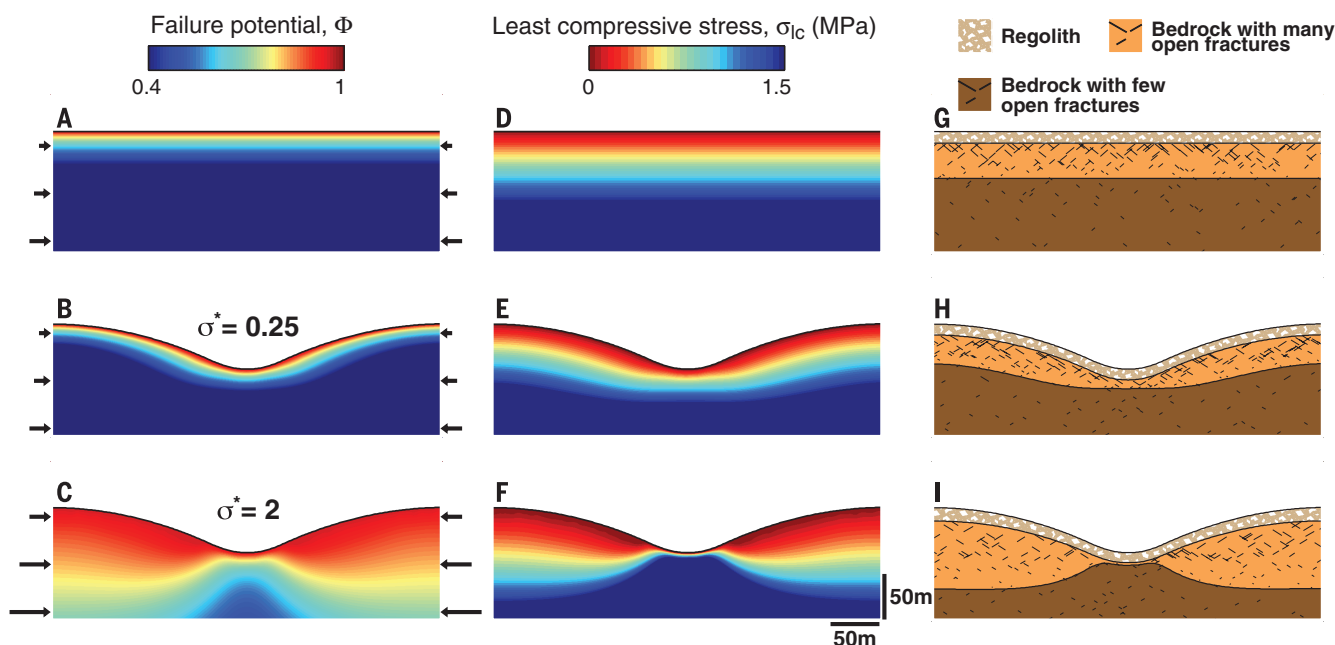


Fig. 1. Hypothesized influence of topography and tectonic stress on weathering zone geometry. (A to C) Cross-sectional plots of failure potential (Φ) computed with a numerical stress model (22). Arrows indicate the magnitude of ambient horizontal compression. (A) shows a horizontal surface under weak compression, (B) shows ridges and valleys under weak compression, and (C) shows ridges and valleys under strong compression. The magnitude of the horizontal compression at the mean elevation of the land

surface is $0.25\rho ga$ in (A) and (B) and $2\rho ga$ in (C), where ρ is rock density, g is gravitational acceleration, and a is the horizontal distance from the valley bottom to the ridge top in (B) and (C). This corresponds to σ^* values of 0.25 in (B) and 2 in (C) (Eq. 1). Topography is horizontally periodic. (D to F) The same scenarios as in (A) to (C), but showing the magnitude of the least compressive principal stress, σ_{lc} . (G to I) Conceptual diagrams of bedrock fracture abundance in the three scenarios.

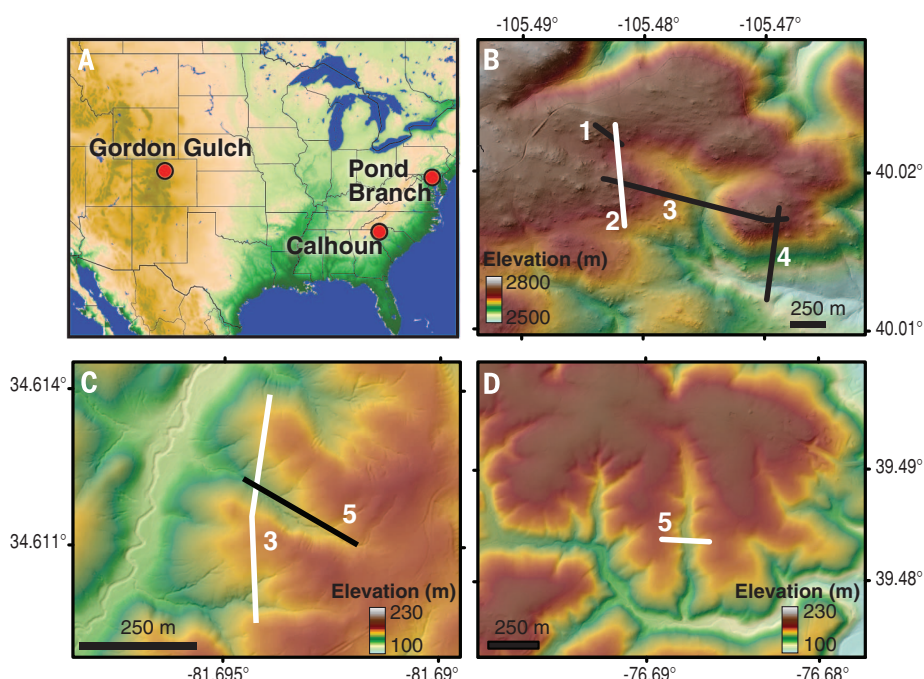


Fig. 2. Maps of study sites. (A) Locations of the three U.S. sites. Colors indicate elevation, with greens being lowest and browns being highest. (B) Gordon Gulch, Colorado. (C) Calhoun, South Carolina. (D) Pond Branch, Maryland. Geophysical survey lines are numbered. White lines mark the transects shown in Fig. 3. Elevation data are from the U.S. National Elevation Dataset and the National Center for Airborne Laser Mapping.

the ambient stress estimates and variations in topography across the sites confirm that these patterns are robust (fig. S3 to S5) (22).

We performed seismic refraction and electrical resistivity surveys along the same transects to investigate whether topographic stresses influence the geometry of the weathered zone (22). The seismic velocity structure reflects variations in rock damage such as chemical weathering and fracturing (28), both of which contribute to an increase in porosity and a decrease in seismic velocity (29). Velocity in fractured rocks can also increase with higher water saturation, which increases the effective bulk modulus of the composite medium (28, 30), or higher confining stress, which closes microfractures formed during the rock's thermal and tectonic history (31). We observed steep velocity gradients with depth, with P -wave velocities (v_p) increasing downward from ~ 0.3 km/s near the surface to ~ 4.0 to 4.5 km/s at 10 to 40 m depth (Fig. 3, G to I), consistent with a transition from soil regolith to unweathered bedrock (28). The rapid increase in velocity with depth is likely also due in part to closure of fractures with increasing confining stress (31). At depths greater than the 4-km/s velocity contour, fractures are likely to be effectively closed, and the velocity gradients are greatly reduced. Borehole observations, including velocity measurements at fine depth increments (fig. S7B) and fractures mapped in optical image logs (fig. S10)

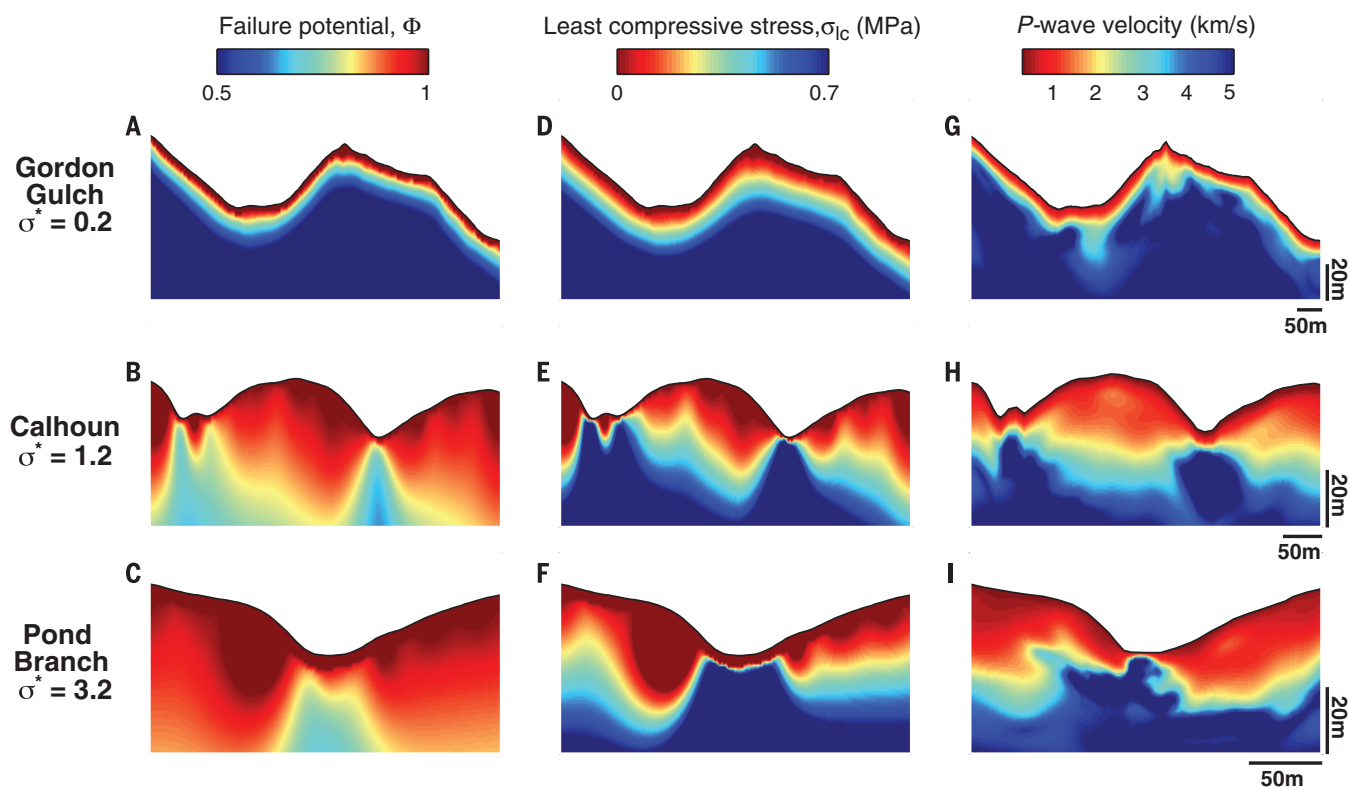


Fig. 3. Comparisons of topographic stress and seismic velocity. Modeled failure potential (A to C), modeled magnitude of the least compressive stress (D to F), and measured P -wave velocity (G to I) for Gordon Gulch [(A), (D), and (G)], Calhoun [(B), (E), and (H)], and Pond Branch [(C), (F), and (I)]. Transect locations are marked in white in Fig. 2. The topographic surface was smoothed for stress calculations (17). Vertical exaggerations are $3.6\times$ at Gordon Gulch, $3.7\times$ at Calhoun, and $2.3\times$ at Pond Branch.

(22), support the interpretation that faster velocities correspond to less abundant open fractures.

The variations in seismic velocity are highly similar in shape to the variations in modeled Φ and σ_{1c} , particularly in the zones of low velocity ($v_p < 4$ km/s; Fig. 3, G to I), which generally correspond to zones of high Φ ($\Phi > 0.75$; Fig. 3, A to C) and low σ_{1c} ($\sigma_{1c} < 0.5$ MPa; Fig. 3, D to F). The 4-km/s velocity contour rises to within 10 m of the surface beneath valley bottoms and plunges to depths of 40 m beneath ridgetops at the sites with strong tectonic compression and $\sigma^* > 1$ (Fig. 3, H and I). This is very similar to the mirror-image pattern of Φ and σ_{1c} contours that is predicted by the stress model (Fig. 3, B, C, E, and F). In contrast to these sites, both the 4-km/s velocity contour and the zones of high Φ and low σ_{1c} roughly parallel the land surface at Gordon Gulch (Fig. 3, A, D, and G), which has weak tectonic compression and $\sigma^* < 1$. The velocity fields contain some fine-scale structures that do not appear in the modeled stress fields, but the stress models do predict some secondary features of the velocity structure. For example, the zones of high Φ and low seismic velocity at Pond Branch are both thicker beneath the right-hand side of the valley bottom (Fig. 3, C and F), and the zone of low σ_{1c} shows this asymmetry for slightly smaller values of σ^* (fig. S4D). The bases of these three zones slope down to the right beneath both ridges at Calhoun (Fig. 3, B, E, and H).

At all three sites, Φ and σ_{1c} correlate well with seismic velocity (fig. S8). The trends of σ_{1c} versus v_p for the three sites are very similar (fig. S8, D to F), whereas the trends of Φ versus v_p vary (fig. S8, A to C), particularly at Gordon Gulch. This may indicate that opening of fractures oriented perpendicular to the σ_{1c} direction is a stronger control on seismic velocity than shear fracturing or sliding at these sites, or it may reflect differences in rock properties among sites that influence the relationship between stress and seismic velocity. In general, the strong correspondence between Φ , σ_{1c} , and seismic velocity at these sites suggests a robust influence of topographic stress on the physical state of weathered bedrock in the subsurface.

We measured electrical resistivity to determine whether the zones of high Φ , low σ_{1c} , and low seismic velocity also have high water

content and more weathered rock. Rocks become less electrically resistive with increasing water saturation, connectedness of pores, and clay content (32). The resistivity images show structures similar to those in the seismic velocity images and reveal additional details about the hydrology of the weathered zone. For example, resistivity at Calhoun increases with depth, and we identified three regions that roughly correlate with the seismic velocity structure (Fig. 4). Where seismic velocities exceed 4 km/s, resistivity is generally high (>4000 ohm-m), consistent with low water content and poorly connected pores in intact bedrock. The upper 10 m of the weathered zone has low resistivity (100 to 700 ohm-m) and velocities < 1.5 km/s, consistent with a highly porous, water-saturated regolith. Between these zones is a zone of intermediate resistivity (700 to 4000 ohm-m) and velocity (1.5 to 4.0 km/s), consistent with fractured, slightly weathered bedrock with intermediate water content. We additionally observed a resistive body roughly 10 m beneath the ridgetop that has higher seismic velocity than the surrounding rock and is in close proximity to a region of locally reduced Φ and elevated σ_{1c} (Fig. 3, B and E).

The geophysical observations support the hypothesis that topographic stress influences bedrock weathering by modifying the abundance of open fractures. This interpretation is consistent with the borehole sonic velocity log at Calhoun (fig. S7B) and the borehole image logs at Gordon Gulch (fig. S10) (22), both of which show lower velocities where fractures are more abundant. However, it is important to consider how factors other than fracture abundance might influence seismic velocity and resistivity. Higher water saturation can elevate seismic velocity, but our resistivity maps show the opposite: a near-surface layer with high water content but lower velocity (Fig. 4). Stress-induced pore pressure variations are also an unlikely explanation for the observed seismic velocity variations, because the velocity field resembles the least compressive stress rather than the mean stress. A more likely scenario is that areas with lower velocities and lower resistivity have experienced a higher degree of chemical weathering. This possibility does not conflict with the interpretation that fractures influence

velocity and resistivity variations; more abundant, open, and connected fractures should promote faster groundwater flow and chemical weathering, which would enhance the connection between topographic stress and bedrock damage.

Our results suggest that topographic perturbations to gravitational and tectonic stress fields control the distribution of open fractures, permeability, and water storage potential in a weathered subsurface zone with a thickness comparable to the topographic relief. We propose that the base of the weathered zone is the depth above which stresses allow abundant fractures to grow or open, providing pathways for water and biota to invade the bedrock and initiate weathering. The stress field thus sets the physical stage for the interacting geochemical (2), hydrological (1), biological (33), and climatic (3) processes that drive weathering and nutrient cycling in Earth's critical zone, which in turn help regulate the global carbon cycle (6). Lateral variations in stress-mediated weathering may also lead to variations in bedrock erodibility and the production rate of mobile soil. Topographic stress may ultimately govern the evolution of landforms through its influence on subsurface weathering and, thus, on erosion at the surface.

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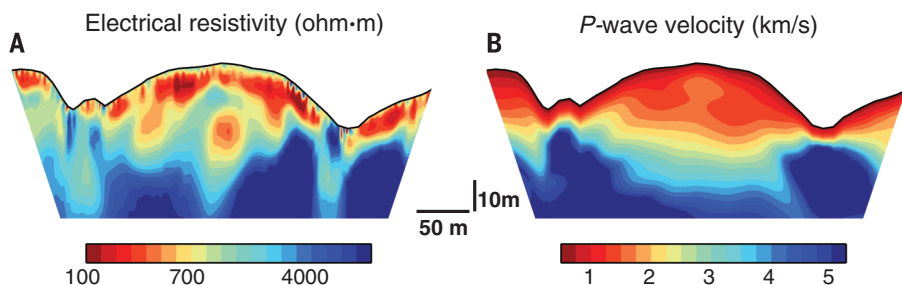


Fig. 4. Electrical resistivity and seismic velocity at the Calhoun site. The transect is the same as in Fig. 3, B, E, and H. Resistivity (A) and P-wave velocity (B) increase with depth, probably reflecting the closing of fractures. Both images show the surface-mirroring pattern predicted by the stress model (Fig. 3, B and E). Vertical exaggeration is 3×.

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SUPPLEMENTARY MATERIALS

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MAGNETISM

Mobile metallic domain walls in an all-in-all-out magnetic insulator

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Magnetic domain walls are boundaries between regions with different configurations of the same magnetic order. In a magnetic insulator, where the magnetic order is tied to its bulk insulating property, it has been postulated that electrical properties are drastically different along the domain walls, where the order is inevitably disturbed. Here we report the discovery of highly conductive magnetic domain walls in a magnetic insulator, Nd₂Ir₂O₇, that has an unusual all-in-all-out magnetic order, via transport and spatially resolved microwave impedance microscopy. The domain walls have a virtually temperature-independent sheet resistance of ~1 kilohm per square, show smooth morphology with no preferred orientation, are free from pinning by disorders, and have strong thermal and magnetic field responses that agree with expectations for all-in-all-out magnetic order.

Magnetic order, in particular antiferromagnetism, often accompanies metal-insulator transitions (MITs), during which metallic materials abruptly become insulating or semiconducting at certain critical temperatures (*1*). Whether metallic behavior can be recovered in these magnetic insulators at magnetic domain walls (DWs), where the order is inevitably disturbed, is a long-standing question that is widely addressed theoretically but remains elusive experimentally (*2–5*). A related but distinct situation is that of conductive ferroelectric DWs in ferroelectric insulators, the discovery of which has opened a broad field of

research dedicated to understanding the fundamental mechanism, as well as making practical DW-based devices (*6–9*). However, among the vast array of magnetic insulators, no conductive magnetic DW has been firmly identified so far. Recently, bulk measurements have provided signatures of DW conduction in the low-temperature insulating phase of Nd₂Ir₂O₇ (*10*), a candidate for the exotic “Weyl semimetal” with an unusual all-in-all-out magnetic order (*11–16*). Here we combine bulk measurements with local-conductivity-measuring microwave impedance microscopy (MIM) to directly resolve highly conductive DWs in Nd₂Ir₂O₇ in real space. These results rule out alternative contributions and thus provide evidence for a realization of conduction due to the discontinuity of magnetic order.

Nd₂Ir₂O₇ is a pyrochlore iridate in which the electronic states near Fermi energy are dominated by *t*_{2g} electrons from Ir atoms (*17*). It undergoes a MIT at a Néel temperature *T*_N ~32 K (Fig. 1A), with a concomitant all-in-all-out (AIAO) magnetic order developing for the Ir magnetic moments (*14, 18, 19*): The Ir moments at the four vertices of each corner-sharing tetrahedron all point either inward or outward in an alternating manner (Fig. 1B). This unusual magnetic order is a ferroic order of magnetic octupole and preserves the symmetry of the underlying lattice (*13*). For a

given lattice, there are two and only two distinct variations of the order: all-in-all-out (AIAO) and all-out-all-in (AOAI). They represent opposite magnetic octupoles and are interchanged with each other by time-reversal transformation; by contrast, their electronic properties should be identical in the absence of external perturbations.

Nonetheless, abnormal conduction can happen at the boundaries between AIAO and AOAI domains (i.e., magnetic DWs), as hinted by macroscopic transport measurements (*10*): The resistance of the same polycrystalline sample at 4.5 K after cooling from above *T*_N in zero field (ZFC or “untrained”) can be more than a factor of 200 smaller than that cooled in a 9-T field (FC or “trained”) (Fig. 1A). One may attribute the extra conduction to DWs, with field cooling resulting in fewer magnetic domains, fewer DWs, and thus higher overall resistance. However, alternative explanations, including history dependence of the bulk and grain boundaries, cannot be ruled out by these macroscopic measurements.

We confirm the existence of highly conductive magnetic DWs by direct imaging with MIM, a scanning probe technique that senses local conductivity by measuring tip-sample impedance at ~1 GHz (*20–22*): More conductive regions screen the microwave electric field better, resulting in a smaller tip-sample impedance. Working at high frequency eliminates the need of a back electrode and a complete current path, making MIM ideal for bulk insulating samples (*23*). Figure 1C is an 18 μm by 18 μm MIM scan of a polished Nd₂Ir₂O₇ polycrystal surface (fig. S1A) at 4.7 K after cooling in zero field (untrained). Smooth curvilinear features that are much more conductive than the bulk exist in all grains, with an apparent width of ~100 nm, similar to the spatial resolution of MIM in this particular experiment (fig. S2). They are continuous within individual grains, show no preferred orientations, and either form closed loops or terminate at the grain boundaries, but they never form vertices. As a result, each grain is divided into regions that can be assigned using only two labels, as expected for AIAO order, which has only two variations. Interestingly, many DWs from adjacent grains are in close proximity at the grain boundaries (yellow arrows in Fig. 1C). Such proximity may facilitate transport across grain boundaries and enhance the DW contribution to the measured conductance in macroscopic polycrystalline samples (*10*). We stress here that the

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grain boundaries themselves are as insulating as the bulk; thus, their contribution to the extra conduction can be ruled out (fig. S1, B and C).

Notably, while the domains are stable against heating to below T_N , they are completely randomized after a thermal cycle to above T_N (Fig. 1D) (24),

indicating that they are tied to the AIAO order and are not pinned by quenched disorders. It also implies that the system randomly evolves into

Fig. 1. Microwave impedance microscopy (MIM) reveals conductive magnetic domain walls in $\text{Nd}_2\text{Ir}_2\text{O}_7$.

(A) Four-terminal resistance of a macroscopic polycrystal taken during zero-field warming after cooling in zero field (untrained) and 9 T field (trained) from 50 K. (B) Illustration of MIM scanning setup on polished polycrystal $\text{Nd}_2\text{Ir}_2\text{O}_7$, showing the spin configuration of AIAO order and its AIAO/AOI variations. Domain walls can exist between the two variations. (C) An $18\ \mu\text{m}$ by $18\ \mu\text{m}$ MIM image of a polished $\text{Nd}_2\text{Ir}_2\text{O}_7$ polycrystal surface, after zero-field cooling from 40 to 4.7 K. A higher MIM signal corresponds to a higher local conductivity. The dotted lines are grain boundaries and the dark spots are voids between grains, which can be identified in higher-temperature scans (fig. S1, B and C). Curvilinear features much more conductive than the bulk are observed in all grains and are identified as AIAO magnetic domain walls. They either form closed loops or terminate at the grain boundaries, sometimes in close proximity (yellow arrows). (D) Same region (with a small offset) after a thermal cycle to 40 K and back to 4.7 K in zero field, showing randomized domains. (E) Same region after a thermal cycle to 40 K and cooling back to 4.7 K in 9 T. Most grains become single-domain, agreeing with transport. Scale bars: $2\ \mu\text{m}$.

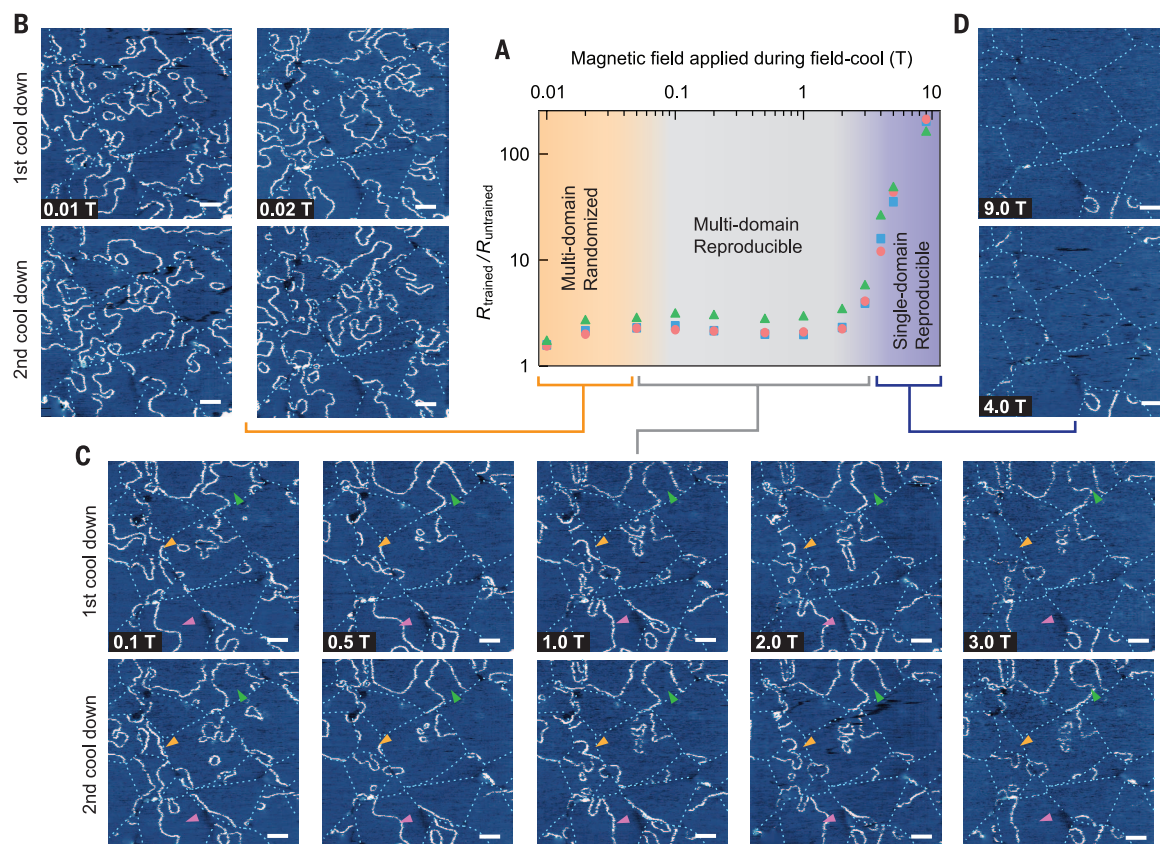
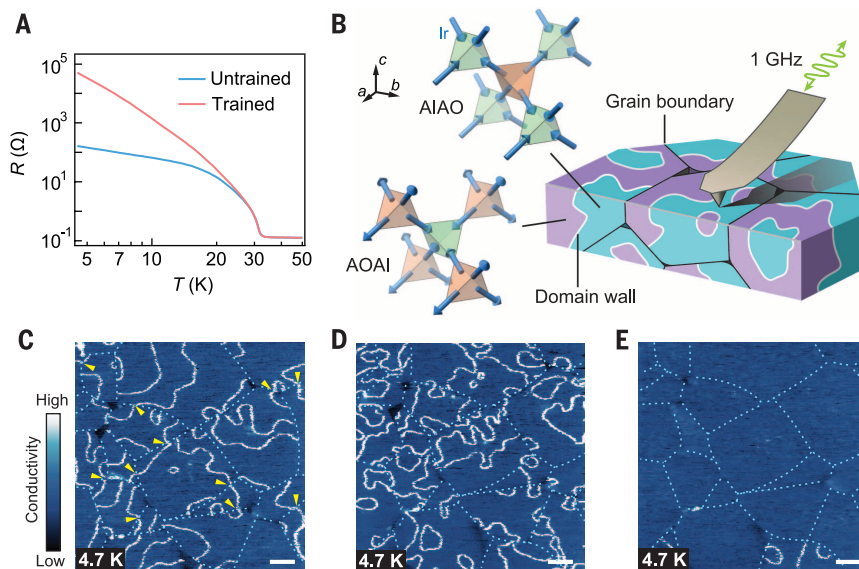


Fig. 2. MIM images for various values of magnetic field applied during cool-down. A multidomain ground state reproducible between thermal cycles is recovered if cooled in 0.1 T or higher. (A) Ratio of final resistance at 4.5 K after cooling in various fields versus cooling in zero field. Data from three samples are shown. (B) MIM images of the same region as in Fig. 1 after two consecutive thermal cycles from 40 to 4.7 K in 0.01 and 0.02 T, showing abundant DWs and

randomized domain configurations between thermal cycles. (C) Same region after cooling in 0.1 to 3.0 T, showing reduced number of DWs and the recovery of a reproducible multidomain ground state. Colored arrows highlight dependence of DW configuration on field magnitude. (D) Same region after cooling in 4.0 and 9.0 T, showing rapid disappearance of DWs in most but not all grains. Scale bars: $2\ \mu\text{m}$.

one of the many near-degenerate multidomain states, with substantial energy barriers in between. Finally, most conductive features disappear after cooling in a 9-T field (trained), in agreement with transport results. The evidence above supports the conductive curves being AIAO magnetic DWs, with conductivity much enhanced in comparison to the bulk.

A multidomain ground state reproducible between thermal cycles is recovered if cooled in a field of ≥ 0.1 T, with an unexpected dependence on the field magnitude (Fig. 2). For cooling in very small fields (≤ 0.02 T), the DWs remain abundant and randomized between thermal cycles as in the ZFC case (Fig. 2B). As the field is increased to ~ 0.1 T, the number of DWs within each grain decreases; in the meantime, DW configuration be-

comes mostly reproducible between thermal cycles, indicating a well-defined multidomain ground state (Fig. 2C). Whereas the number of DWs remains largely stable up to 3 T (corresponding to the plateau of FC/ZFC resistance ratio in Fig. 2A), the detailed DW configuration changes unmistakably as a function of field magnitude (compare, for example, regions marked by colored arrows in Fig. 2C). This indicates competition of AIAO and AOAI coupled to local degrees of freedom—for example, strain via the predicted magnetostriction effect (13). Above ~ 3 T, a single-domain ground state quickly becomes favorable (Fig. 2D), likely due to the field response of the much larger Nd moments, coupled to Ir moments through f-d coupling (12, 14, 15, 25), and the resistance increases rapidly (Fig. 2A). Nevertheless, a few grains

remain multidomain even if cooled in 9 T, the origin of which can be well explained with crystal orientation dependence, as discussed below.

The field response of the DWs at a fixed temperature demonstrates the expected crystal orientation dependence of AIAO magnetic order. Figure 3A shows the evolution of DWs as an out-of-plane magnetic field is increased from 0 to 9 T, at 4.7 K after ZFC. Figure 3C shows the out-of-plane crystal orientation of the same area obtained with x-ray microdiffraction (μ XRD) (26). DWs disappear abruptly at grain-specific critical fields that depend on the crystal orientation. The first grain to become single-domain at 3 T (blue triangle in Fig. 3A) has an out-of-plane direction closest to [111] (dark blue color in Fig. 3C). Most other grains subsequently transition between 4

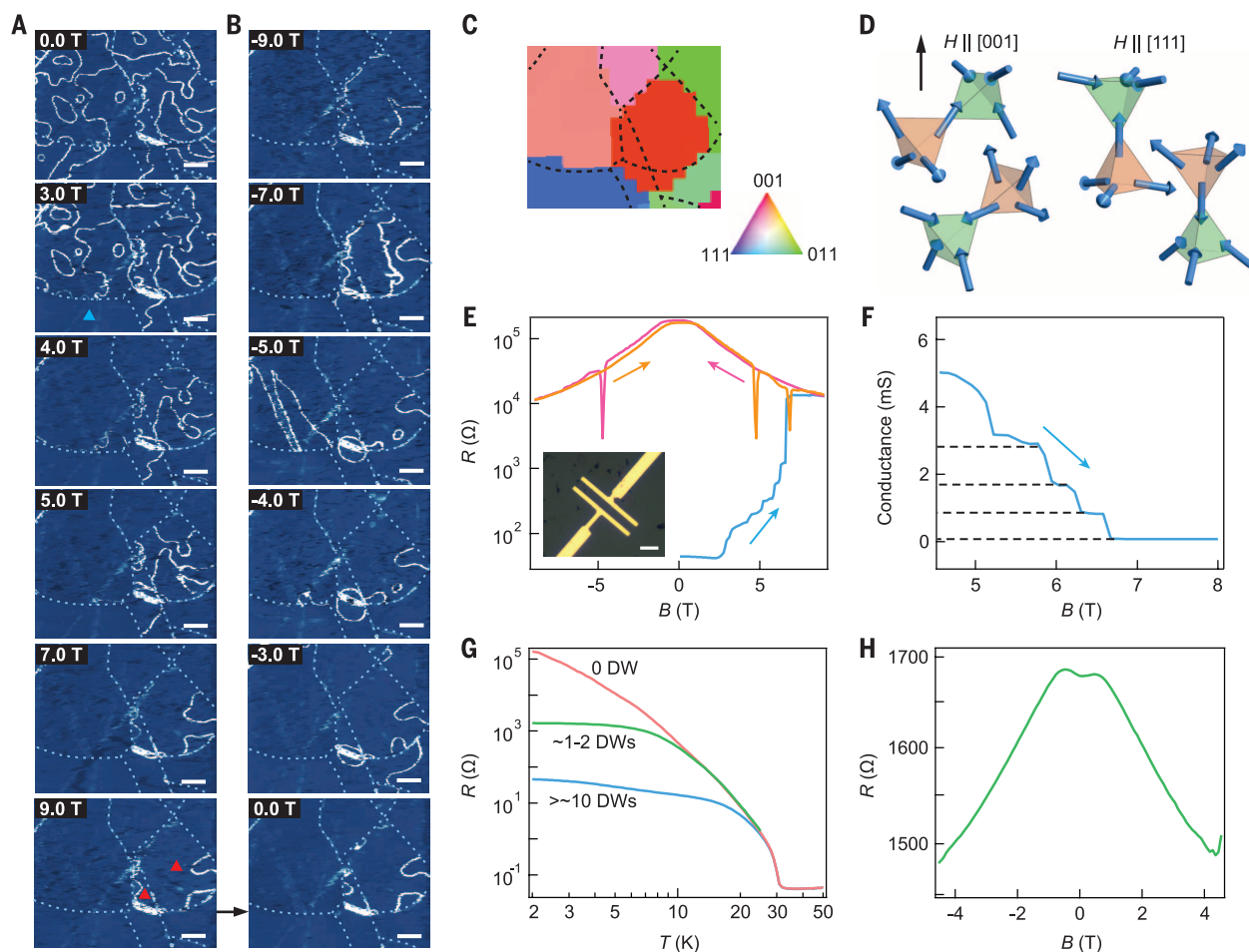


Fig. 3. Field-dependent domain evolution at low temperature and DW sheet resistance measured with microelectrodes. (A) Field dependence of DWs at 4.7 K. The first grain to become single-domain is marked by a blue triangle, and the two grains that remain multidomain at 9 T by red triangles. (B) The same region during subsequent field sweeps back to 0 T and then to -9 T, showing transient DW formation during domain reversal. (C) Out-of-plane crystal orientation of the same area obtained with μ XRD, as indicated by the tricolor map. The blue and red grains in (A) have an out-of-plane orientation closest to [111] and [001], respectively. (D) Illustration of field-induced spin canting with a field along the [111] and [001] direction for AIAO and AOAI configurations: The largest difference is generated for the [111] case, while the effects are equivalent for the [001] case, consistent with data in (A) and (C).

(E) Typical resistance versus magnetic field of a microelectrode device (inset) at 2 K when sweeping the field from 0 to 9 T (blue), 9 to -9 T (magenta), and -9 to 9 T (orange) after a ZFC. Discrete resistance jumps and sharp resistance minima are shown. Scale bar (inset): $5 \mu\text{m}$. (F) The initial sweep (blue) in (E) converted to conductance, showing staircase-like conductance drops. Sheet resistance of DWs averaged over mesoscopic lengths is thus estimated to be ~ 1 kilohm/sq. (G) Temperature dependence of resistance for various initial states taken during zero-field warming, showing the virtually temperature-independent conduction property of a few DWs. (H) Magneto-resistance of a few DWs at 2 K. The contribution from bulk magnetoresistance is negligible and is subtracted as a parallel resistor. Scale bars (A and B): $2 \mu\text{m}$.

and 7 T. The two grains that remain multidomain even at 9 T (red triangles in Fig. 3A) are closest to [001] (red color in Fig. 3C). This is in excellent agreement with expectations from the symmetry of AIAO order: The magnetic field along [111] gives rise to the largest difference in spin canting between AIAO and AOAI, whereas the field along [001] gives rise to equivalent perturbations in the two variations, connected by a simple 90° rotation along [001] (Fig. 3D) (13). Therefore, the critical field should be smallest when applied in the [111] direction and largest in the [001] direction, regardless of the detailed mechanism. The grain that remains multidomain in Figs. 1 and 2 is also close to [001], as confirmed by μ XRD data (fig. S3). These observations further corroborate the identification of the conductive curves as AIAO DWs.

The sheet resistance of a single DW averaged over mesoscopic lengths is ~ 1 kilohm/sq. This value was obtained by fabricating microelectrodes on the sample surface to measure transport directly across a few grains [Fig. 3E (inset) and fig. S4A]. Figure 3E shows a typical resistance-versus-magnetic-field plot of such devices at 2 K following a ZFC. During the initial field sweep from 0 to 9 T (blue trace), resistance increases in discrete steps, reflecting the abrupt disappearance of DWs in each grain contacted by electrodes (Fig. 3A). By converting resistance into conductance, we notice that the decreases in conductance associated with each jump are markedly similar, at ~ 1 mS (Fig. 3F and fig. S4B). Assuming one or two DWs in each grain and an average effective aspect ratio on the order of 1 (fig. S4A), we conclude that the sheet resistance of the AIAO DW averaged over mesoscopic lengths is ~ 1 kilohm/sq. This value is consistent with the three-dimensional-averaged DW conductivity obtained by terahertz spectroscopy measurements (10), assuming an average DW spacing of 2 μ m.

The sheet resistance of DWs has very weak temperature dependence at low temperatures and shows substantial negative magnetoresistance. We first develop a method to reliably prepare a few DWs across the electrodes by capturing transient DW formation. Following the staircase-like trace during the initial field sweep, sharp resistance minimums occur for subsequent sweeps (magenta and orange trace in Fig. 3E), because of transient DW formation during the rapid grain-wise domain reversals (from AIAO to AOAI, or vice versa), as also seen in MIM images (Fig. 3B). These sharp reversals ensure that if the field sweep is stopped immediately when a resistance minimum occurs, only one or two DWs will typically exist across the electrodes. We find that these artificially created DWs are stable against temperature and magnetic field sweeps, as long as we stay below T_N and the critical field. The few DWs obtained this way show ohmic behavior (fig. S5) and a virtually temperature-independent sheet resistance until overwhelmed by bulk conduction at high temperatures (Fig. 3G). Indeed, the resistance of multiple DWs increases by only $\sim 10\%$ when cooling from 0.6 K to 35 mK (fig. S6),

indicating a gapless electronic structure that is moderately localized owing to, for example, the slowly varying DW orientation. The gapless nature of the DWs is further corroborated by thermal power measurements (fig. S7). The DW resistance shows substantial negative magnetoresistance for fields up to ± 4 T (Fig. 3H). A small minimum near zero-field resembles weak antilocalization, as commonly seen in materials with strong spin-orbit coupling (27).

The metallic DWs result from mid-gap states that are extended within the DW plane but are localized perpendicular to it. Such “defect states” are generally present, theoretically, if the magnetically disordered state is metallic and the disturbance to the order parameter is sharp (2–6, 28). Indeed, metallic DWs are not observed in other $\text{Ln}_2\text{Ir}_2\text{O}_7$ (where Ln is Sm, Eu, etc.) with similar magnetic order: Unlike $\text{Nd}_2\text{Ir}_2\text{O}_7$, they are all semiconducting instead of metallic above the magnetic transition temperature (12). The highly anisotropic AIAO order is also expected to give rise to sharp DWs in $\text{Nd}_2\text{Ir}_2\text{O}_7$: The spins are locked firmly to [111] and equivalent crystal orientations via spin-orbit coupling and cannot rotate continuously across magnetic domains (15). The resulting sharp DWs represent an abrupt disturbance of magnetic order parameter and can host mid-gap states well separated from bulk states. The picture of magnetic-superlattice-driven MIT (Slater insulator) is not applicable here because AIAO order preserves the symmetry of the underlying pyrochlore lattice (29).

In the particular case of iridates, the above generic picture may be captured by the interface states near an exotic “Weyl semimetal” phase. The AIAO order is predicted to host a “Weyl semimetal” phase near the MIT with “Weyl fermions” in the bulk and “Fermi arc” states at the surfaces (11), as well as interfaces between AIAO and AOAI domains (30). At temperatures much lower than the transition, the bulk becomes insulating as the “Weyl fermions” annihilate and the “Fermi arc” states disappear at the surfaces. Nonetheless, mid-gap states are shown to survive at the magnetic DWs (30). Our observation—namely, of metallic DWs and insulating surfaces and grain boundaries—is consistent with the prediction of this theory. A conclusive confirmation, however, requires more systematic studies.

The highly conductive, mobile DWs show no preferred orientation, are free from pinning by disorders, and can be easily manipulated via heat, magnetic field, and probably strain and geometry. They provide an excellent platform, scientifically, for studying exotic emergent phenomena at interfaces, and practically, for DW-based memory devices that can be read electronically without relying on magnetic junctions (31). Similar properties may be present in other materials with a true MIT and a highly anisotropic magnetic order, commonly seen in heavy-element materials with large spin-orbit coupling.

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Figs. S1 to S8

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SUPERCONDUCTIVITY

Quantum Griffiths singularity of superconductor-metal transition in Ga thin films

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The Griffiths singularity in a phase transition, caused by disorder effects, was predicted more than 40 years ago. Its signature, the divergence of the dynamical critical exponent, is challenging to observe experimentally. We report the experimental observation of the quantum Griffiths singularity in a two-dimensional superconducting system. We measured the transport properties of atomically thin gallium films and found that the films undergo superconductor-metal transitions with increasing magnetic field. Approaching the zero-temperature quantum critical point, we observed divergence of the dynamical critical exponent, which is consistent with the Griffiths singularity behavior. We interpret the observed superconductor-metal quantum phase transition as the infinite-randomness critical point, where the properties of the system are controlled by rare large superconducting regions.

Quenched disorder can have profound effects on phase transitions. One of the most striking is the so-called Griffiths singularity, which appears in some systems as a consequence of the formation of rare ordered regions (1–5). In those systems, the critical point in the presence of disorder was predicted to show exponential instead of power-law correlations and a divergent dynamical critical exponent $z \rightarrow \infty$ (1–5). This Griffiths singularity behavior has been theoretically studied in lattice models of various spatial dimensions (6–9), and experimental evidence in three-dimensional magnetic metals has been reported (10–12). However, the most prominent feature of the Griffiths singularity, the divergence of the dynamical critical exponent, has been challenging to observe experimentally in two-dimensional (2D) systems.

Here, we show that the superconductor-metal transitions (SMTs) in Ga films display behaviors that are consistent with the quantum Griffiths singularity. Superconductor-insulator transitions (SITs) or SMTs have been observed in several quasi-2D disordered superconducting systems (13–16). These systems are expected to go through a zero-temperature quantum phase transition (QPT) by tuning the film thickness or the applied magnetic field B (16, 17). The zero-temperature quantum critical point (QCP) and its properties can be obtained from scaling analysis of finite-temperature data. In particular, the crossing of

$R_S(B)$ curves measured at different temperatures (where R_S denotes sheet resistance) is considered as the evidence of such a QPT at zero temperature, and the crossing point is identified as the critical magnetic field B_C (16, 18). Recently, two crossing points of $R_S(B)$ curves were observed in superconducting oxide interfaces and copper oxide superconductor films, and were interpreted as two quantum critical behaviors of a SMT (19) or a two-stage, magnetic field-tuned SIT (20), respectively. These results suggest that our understanding of SMTs leaves room for improvement.

Ga films with a nominal thickness of three monolayers (3 ML) were epitaxially grown on a 3- μm GaN(0001) substrate in an ultrahigh-vacuum molecular beam epitaxy (MBE) chamber (21). Scanning tunneling spectroscopy (STS) measurements at 3.00 K without external magnetic field revealed an energy gap (differential conductance dI/dV versus voltage V) with two pronounced coherence peaks, whose temperature dependence can be fitted to the Dynes function

$$\frac{dI}{dV} \propto \int_{-\infty}^{+\infty} \text{Re} \left[\frac{|E - i\Gamma|}{\sqrt{(E - i\Gamma)^2 - \Delta^2}} \right] \times \left(\frac{\exp[(E + eV)/kT]}{kT \{1 + \exp[(E + eV)/kT]\}^2} \right) dE \quad (1)$$

(22, 23), as shown in fig. S1B (here, Γ is a broadening factor, k is the Boltzmann constant, E denotes energy, and e is the elementary charge) (21). The STS curves acquired at various temperatures further demonstrate that the observed gap Δ is a superconducting gap; the fits yield $\Delta(0) = 1.080$ meV, $T_C = 5.11$ K, and Bardeen-Cooper-Schrieffer ratio $2\Delta(0)/kT_C = 4.9$ (fig. S1, C and D). A series of STS (dI/dV) data obtained along an 8-nm line indicated spatially

homogeneous superconductivity under zero external magnetic field (fig. S1, E and F).

For ex situ transport measurements, amorphous Si film (~140 nm thick; fig. S2) was deposited on the 3-ML Ga films in the MBE chamber as a protection layer. The Si capping layer can also act as the immobilization support of Ga films. Standard four-electrode transport measurements of the 3-ML Ga films (Si/Ga/GaN/AlN/Al₂O₃) were carried out (21). As a function of temperature at zero magnetic field, the value of R_S manifests a zero-resistance transition temperature $T_C^{\text{zero}} \approx 3.62$ K (Fig. 1A). T_C^{zero} is identified as R_S falls below the instrument resolution limit (here, ± 0.01 ohms). Figure 1B reveals the $R_S(T)$ properties at various magnetic fields (the magnetic field is perpendicular to Ga films). With increasing magnetic field, the superconducting transition shifts monotonically to lower temperatures. Intriguingly, as the magnetic field continues to increase, the quasi-2D superconductor gradually changes to a weakly localized metal. At a magnetic field of 2.270 T, the two regimes separate and the corresponding $R_S(T)$ is approximately a constant at low temperature. Magnetic fields up to 8.000 T were applied to the film, and the $R_S(B)$ curves cross each other at ~2.235 T, with an uncertainty of 0.125 T (Fig. 1C).

$V(I)$ characteristics were measured on the 3-ML Ga film from 2.00 to 8.00 K in the absence of magnetic field (Fig. 1D). The critical current I_C increases as the temperature decreases, reaching 5 mA at 2.00 K. At each temperature, when the current is higher than the corresponding I_C , a power-law dependence of $V \sim I^\alpha$ is observed, where the exponent α , as reflected in the slopes, changes systematically as a function of temperature (Fig. 1D, inset), indicating a Berezinski-Kosterlitz-Thouless (BKT)-like transition in 2D superconductors (24). The exponent α deviates from 1 with decreasing temperature and approaches 3 at 3.72 K (identified as T_{BKT}), which is close to the value of $T_C^{\text{zero}} \approx 3.62$ K. When the current exceeds I_C for the lowest measured temperature, the $V(I)$ curves merge, presenting a linear ohmic law with a resistance corresponding to the normal resistance. To systematically study the details near the superconductor-metal phase boundary, we measured the same sample in a Dilution Refrigerator MNK126-450 system (Leiden Cryogenics BV) in an ultralow-temperature environment. The 3-ML Ga film exhibited crossover behavior between 0.010 K and 0.50 K, similar (fig. S3A) to that found in a higher-temperature regime ($T \geq 1.90$ K) in a PPMS-16T system (Quantum Design) (Fig. 1C). By carefully measuring the magnetoresistance at temperatures between 0.025 K and 2.80 K, we identified a series of similar crossing points, which form a continuous line of SMT “critical” points (Fig. 2). The inset of Fig. 2 shows this crossover boundary in a B - T plot; crossing points of $R_S(B)$ curves at every two adjacent temperatures are shown as black dots. The blue line (1.00 to 2.75 K) is the fitting curve according to Werthamer-Helfand-Hohenberg theory (25). However, the expression cannot fit the ultralow-temperature

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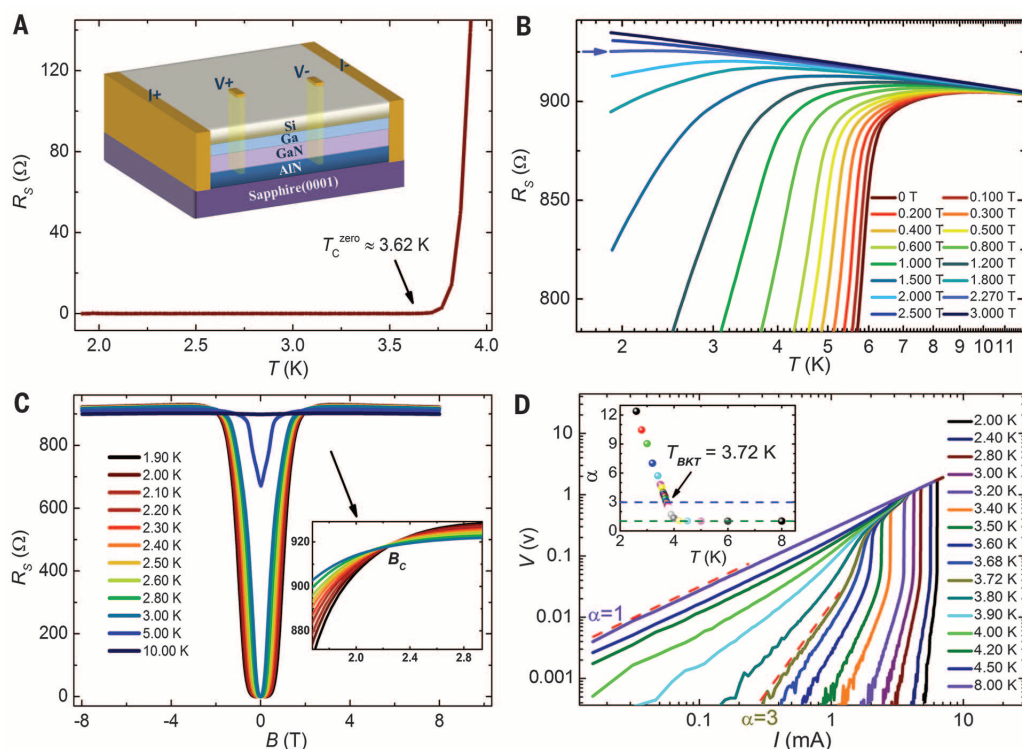


Fig. 1. Transport properties of 3-ML Ga film. (A) $R_S(T)$ curve at zero magnetic field. The zero-resistance transition temperature T_C^{zero} marked by an arrow is 3.62 K. The inset is a schematic structure for the four-electrode transport measurements. (B) $R_S(T)$ behavior measured under various magnetic fields up to 3.000 T. (C) R_S as a function of magnetic field at different temperatures ranging from 1.90 to 10.00 K. Inset: Close-up of the same data near the crossing region (around characteristic magnetic field $B_C = 2.235 \pm 0.125$ T). (D) $V(I)$ characteristics at various temperatures plotted on a logarithmic scale ranging from 2.00 to 8.00 K at $B = 0$ T. The two dashed red lines labeled $\alpha = 1$ and $\alpha = 3$ correspond to $V \approx I$ and $V \approx I^3$ dependences, respectively. Inset: The variation of exponent α as a function of temperature is extracted from the power-law fittings in (D); $T_{BKT} = 3.72$ K is defined by $\alpha = 3$.

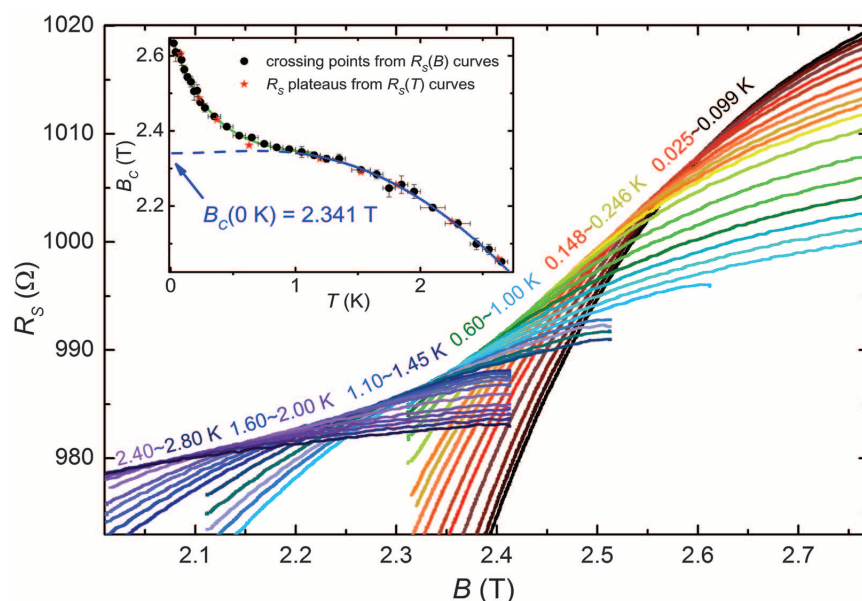


Fig. 2. Magnetoresistance isotherms. The magnetic field dependence of R_S at various temperatures ranging from 0.025 K to 2.80 K, with the same sweep direction and the same sweep rate, exhibits an SMT region. Inset: the critical magnetic fields $B_C(T)$ extracted from Fig. 2. Crossing points of $R_S(B)$ curves at every two adjacent temperatures are denoted as black dots on the transition boundary; the red stars come from the temperature plateaus on $R_S(T)$ curves at given magnetic fields in fig. S4. The blue line is the fitting curve using Werthamer-Helfand-Hohenberg theory, $\ln(T_0/T) = \frac{1}{2}\psi\{\frac{1}{2} + [(aB + ibB)/(2\pi T/T_0)]\} + \frac{1}{2}\psi\{\frac{1}{2} + [(aB - ibB)/(2\pi T/T_0)]\} - \psi(\frac{1}{2})$ for the temperature range 1.00 to 2.75 K, with ψ denoting the digamma function, $a = 0.24$ T⁻¹, $b = 0.29$ T⁻¹, and $T_0 = 6.10$ K. The green line and the dashed blue line (below 1.00 K) are guides to the eye.

data, and the zero-temperature critical magnetic field of SMT from this fitting [$B_C(0$ K) = 2.341 T] is far below the extrapolation of ultralow-temperature data ($B_C^* = 2.639$ T).

We used scaling analysis similar to previous experimental work to analyze this exotic phenomenon with multiple crossing points (19, 26). The sheet resistance of a 2D system can be described

by the phenomenological scaling law (16, 27): $R_S(\delta, T) = R_C \cdot F(\delta T^{-1/z})$, where $\delta = B - B_C$ is the deviation from the critical magnetic field, R_C is the critical sheet resistance, F is an arbitrary function with $F(0) = 1$, ν is the correlation length exponent, and z is the dynamical critical exponent (27–29). A small critical transition region formed by several adjacent $R_S(B)$ curves can be approximated by one “critical” point (B_C, R_C). For instance, $B_C = 2.626$ T ($R_C = 1009.82$ ohms) is an approximate crossing point for a transition region of three $R_S(B)$ curves of 0.025 K, 0.035 K, and 0.053 K (fig. S5A). We define $R_S(\delta, T_0) = R_C \cdot F(\delta)$ for the lowest temperature T_0 , and rewrite the equation above as $R_S(\delta, T) = R_C \cdot F(\delta \cdot t) = R_S(\delta \cdot t, T_0)$, where the unknown parameter $t \equiv (T/T_0)^{-1/z}$ can be determined at each temperature T by performing a rescale of the δ axis of resistance curves $R_S(\delta, T)$ to match $R_S(\delta, T_0)$ (fig. S5A). The effective “critical” exponent $z\nu$ for the temperature regime 0.025 to 0.053 K is then determined to be 8.07 from the definition $t \equiv (T/T_0)^{-1/z\nu}$ (fig. S5B, inset). Nine representative crossing points on the $R_S(B)$ critical boundary were selected (figs. S5 and S6), and the resulting magnetic field and temperature dependence of the effective “critical” exponent $z\nu$ are summarized in Fig. 3 and fig. S7, respectively. Generally, the real $T = 0$ critical exponent can be obtained by taking the asymptotic value of finite-temperature effective “critical” values when temperature approaches zero. However, unlike normal cases, the effective “critical” exponent $z\nu$ grows rapidly with decreasing temperature in the ultralow-temperature regime and approaches infinity with the field approaching B_C^* and the temperature tending to zero.

The observed critical magnetic field boundary behavior was confirmed on another 3-ML Ga film (Si/Ga/GaN/AlN/Al₂O₃, $T_C^{\text{zero}} \approx 2.07$ K) grown under conditions identical to those described above (fig. S8).

The physical origin of the observed low-temperature behavior can be understood in the context of SMT (30–32). Our experimental data support the existence of unpaired normal electrons around the phase transition, which originates from the pair-breaking mechanism as a result of dissipation effect (33, 34). We assume a direct QPT from a superconducting phase to a metallic phase, where the metallic phase means that $k_F \ell \gg 1$ with critical resistance $R_C \ll \hbar/e^2$ and single-particle localization effects are mostly unobservable (here, k_F is the Fermi momentum, ℓ is the mean free path, and $\hbar$ is the Planck constant) (35). The crossover boundary is defined by the turning point of $R_S(T)$, where $dR_S(T)/dT$ changes sign for a given magnetic field. The destruction of superconductivity results from the interplay of quantum fluctuation, quenched disorder, and thermal fluctuation, which leads to a complex B - T phase diagram (36–38). In conventional theory of homogeneous type II superconductors, the Abrikosov vortex lattice forms in the region of $B_1(T) < B < B_2(T)$, characterized by a continuous phase transition at the upper critical field $B_2(T)$ (39, 40). Our observations deviate from this prediction in the low-temperature regime and show an anomalous upturn of upper critical field as $T \rightarrow 0$, as shown in the inset of Fig. 2. We attribute these apparent deviations to the quenched disorder effect on the phase transitions in type II superconductors. In clean systems, thermal fluctuations give rise to phonon-like excitations of the vortex lattice, and when thermal fluctuations become sufficiently large, the system is expected to melt into the vortex liquid phase. This 2D vortex lattice melting transition is characterized by the melting temperature T_M (Fig. 4) (36). However, the long-range order of a vortex lattice is also unstable against the fluctuations caused by quenched disorder. When the temperature decreases to the $T < T_M$ regime, the effect of quenched disorder, with characteristic length L_P , overtakes the thermal fluctuation effect and produces a vortex glass-like phase on length scales greater than L_P (41). This vortex glass-like phase consists of spatially separated superconducting regions, where the local upper critical field exceeds the average mean-field value $B_2(T)$ (Fig. 4). In the ultralow-temperature regime, these separate superconducting islands—namely, the rare regions—can couple via long-range Josephson coupling and manifest global superconductivity in the system. These rare regions of superconductivity not only can cause the peculiar upward turn of upper critical field as $T \rightarrow 0$ but also may exhibit unusual scaling behavior when approaching the field-induced QCP with quenched disorder.

We next investigated the scaling behavior of the QPT (27, 28). Note that quenched disorder is identified as a determining factor in the destruction of a clean QCP. Thus, we speculate that the strong randomness, caused by quenched disorder in the

low-temperature limit, also plays an essential role in the rapidly varying fitting exponent $z\nu$ upon approaching the field-induced QCP with quenched disorder. The stability of a clean QCP with critical exponent ν is commonly determined by the Harris criterion, as applied to analysis of the tendency of average disorder strength under coarse graining (3, 42). In a d -dimensional system with $d\nu < 2$, the disorder strength increases under coarse graining, and the clean critical behavior is changed (3). Moreover, a recent scaling study draws a sweeping conclusion: If the clean QCP violates the Harris criterion, the dynamical exponent z diverges when approaching the critical point, indicating an unusual activated scaling behavior (43). Thus, be-

cause of the existence of rare regions, the Harris criterion is insufficient to characterize the QPT in disordered systems (2, 42). Such rare regions can be locally ordered into one phase and can cause singular thermodynamic properties not only at the critical point but also in its vicinity, namely, the Griffiths singularity (2). One representative example is the quantum random transverse field Ising model (RTFIM), where the magnitude of inhomogeneity grows without limit under coarse graining (4, 5). This system exhibits quantum Griffiths singularity with unusual critical behaviors: the activated scaling with continuously varying dynamical exponent z when approaching the infinite-randomness QCP (5–9).

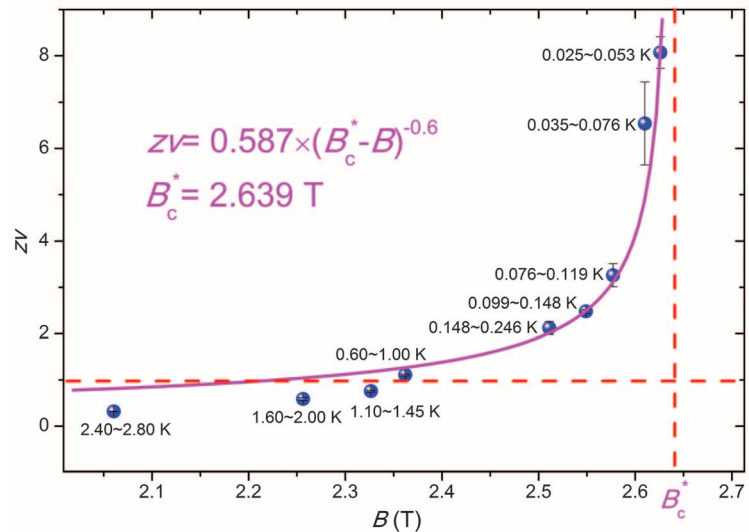


Fig. 3. The activated quantum scaling behavior: Exponent $z\nu$ as a function of magnetic field B .

When approaching the zero-temperature limit, $z\nu$ from figs. S5 and S6 rapidly increases with no sign of saturation. The magenta line shows a fitting based on the activated scaling law.

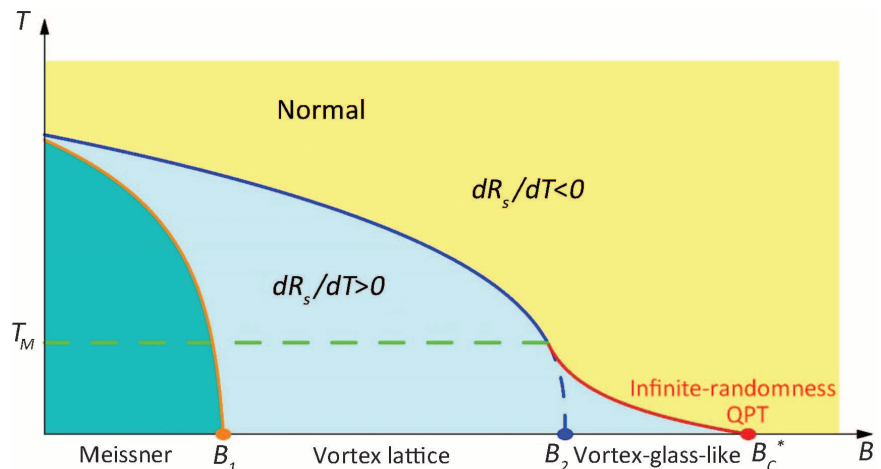


Fig. 4. Sketch of the B - T phase diagram of 2D SMT with quenched disorder. B_2 is the upper critical field and would be the QCP for a clean system. When temperature decreases to $T < T_M$, quenched disorder overtakes thermal fluctuation and gives rise to a vortex glass-like phase. Such a vortex glass-like phase disappears at the infinite-randomness QCP B_c^* . Rare regions of superconducting islands emerge and result in activated scaling behavior around such an infinite-randomness QCP B_c^* .

This activated scaling behavior is consistent with our observation of rapidly diverging exponent $z\nu$ in the vicinity of the field-induced QCP with quenched disorder. To test this paradigm, we turn to the specific analysis of SMT. According to the pair-breaking scenario of SMT, bosonic cooper pairs can be overdamped into normal single-particle excitations, which results in the quantum SMT at $T = 0$ with the clean critical exponent $\nu = 1/2$ (34). Thus, in the 2D system with SMT, the violation of the Harris criterion (with $d\nu = 1$) will most likely lead to the infinite-randomness QCP. Indeed, previous theoretical investigations revealed that the quenched disorder markedly changes the scaling behavior of SMT and results in activated scaling identical to the RTFM (44–47). On this basis, we can fit the experimental results of $z\nu$ by the activated scaling law $z\nu \approx C|B - B_c^*|^{-\nu\psi}$ with constant C and the 2D infinite-randomness critical exponents $\nu \approx 1.2$ and $\psi \approx 0.5$ (8, 9), and the good agreement between our observation and the theoretical expectation strongly supports the existence of infinite-randomness QCP (Fig. 3).

Our findings are summarized by the schematic phase diagram in Fig. 4. When approaching the infinite-randomness QCP at B_c^* , the quenched disorder leads to two correlated phenomena: (i) In the zero-temperature limit, the vortex lattice deforms into a vortex glass-like phase on a length scale $L > L_p$; (ii) because of the transformation into the vortex glass-like phase, rare regions of inhomogeneous superconducting islands gradually emerge in the $B > B_2$ regime and manifest activated scaling behavior, namely, the quantum Griffiths singularity (Fig. 3). One question remains as to why this activated scaling feature has not yet been observed in SMT in previous studies. We attribute this absence to the instability of the vortex glass-like phase under thermal fluctuation. The quenched disorder will play a dominant role for length scales $L > L_p$. Thus, in the high-temperature regime, thermal fluctuations smear the inhomogeneity caused by quenched disorder, and rare regions hardly exist. Near zero temperature, the impact of quenched disorder overtakes thermal fluctuation, which results in the emergence of rare regions around the infinite-randomness QCP (Fig. 4). On the basis of these considerations, we speculate that the activated scaling feature can only be observed under extremely low temperature, which is the case in our study.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/350/6260/542/suppl/DC1
Materials and Methods
Figs. S1 to S8
References (48, 49)

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ECONOMICS

Peer effects on worker output in the laboratory generalize to the field

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We compare estimates of peer effects on worker output in laboratory experiments and field studies from naturally occurring environments. The mean study-level estimate of a change in a worker's productivity in response to an increase in a co-worker's productivity (γ) is $\hat{\gamma} = 0.12$ (SE = 0.03, $n_{\text{studies}} = 34$), with a between-study standard deviation $\tau = 0.16$. The mean estimated $\hat{\gamma}$ -values are close between laboratory and field studies ($\hat{\gamma}_{\text{lab}} - \hat{\gamma}_{\text{field}} = 0.04$, $P = 0.55$, $n_{\text{lab}} = 11$, $n_{\text{field}} = 23$), as are estimates of between-study variance τ^2 ($\hat{\tau}_{\text{lab}}^2 - \hat{\tau}_{\text{field}}^2 = -0.003$, $P = 0.89$). The small mean difference between laboratory and field estimates holds even after controlling for sample characteristics such as incentive schemes and work complexity ($\hat{\gamma}_{\text{lab}} - \hat{\gamma}_{\text{field}} = 0.03$, $P = 0.62$, $n_{\text{samples}} = 46$). Laboratory experiments generalize quantitatively in that they provide an accurate description of the mean and variance of productivity spillovers.

Laboratory experiments in the social sciences are valuable for understanding human behavior in controlled environments (1, 2). However, there is an active debate about the extent to which these experiments have external validity. In an influential paper, Levitt and List have questioned whether findings in laboratory studies generalize in the real world, arguing that “because the lab systematically differs from most naturally occurring environments...

experiments may not always yield results that are readily generalizable” (3). Indeed, on the surface, laboratory experiments appear quite different than natural settings. Subjects tend to be

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students, and the controlled setting may appear artificial in relation to actual workplaces. In spite of the importance of laboratory studies in the social sciences, and of the many articles engaging in this debate (4–7), to our knowledge there has not been a systematic comparison of the same economic parameter estimated in laboratory experiments and field studies using more than a small number of studies.

We exploited the relative abundance of estimates for one particular parameter—the estimated spillover effect of worker productivity on the productivity of co-workers—in order to compare estimates from laboratory experiments with those from field studies that use data from naturally occurring environments. This parameter, which we denote γ , is useful for understanding a variety of economic phenomena, including wage setting, economic growth, the social returns to human capital investment, the effects of immigration, the optimal assignment of workers to teams, and agency problems. Over the past 15 years, there have been more than 34 studies seeking to estimate γ in a diverse set of occupations (8–41), including fruit pickers (11), supermarket cashiers (31), physicians (17), sales teams (18), and scientists (40). The large number of estimates of γ in the literature presents an opportunity to directly compare the findings of laboratory experiments and field studies.

We constructed a database of γ estimates using inclusive criteria (42). We included a study in the database if the paper contains an estimate of the effect of the productivity of a worker or a group of workers on another worker or group of workers, and the paper interprets the estimate as a workplace-productivity peer effect. We included laboratory experiments meant to simulate workplace environments. Estimates from both published and working papers were included to mitigate possible publication bias. This yielded papers in traditional field settings with observable productivity data but also included sports studies (25, 26, 41) and studies estimating spillovers by using administrative earnings data (12, 19, 35). Several studies that estimate worker peer effects (43–46) do not include all of the information required to construct comparable, variance-weighted estimates and were therefore not included. After this screen, our database contains estimates from 34 papers, of which 23 are field studies and 11 are laboratory experiments (table S1). We call this the study-level database. In this database, 50% of the studies are published (Table 1).

Studies often include several estimates from different methodologies, specifications, and/or samples. If multiple methodologies were used, we selected estimates using the methodology the authors explicitly stated as preferred. If the authors did not explicitly state their preferred methodology, we selected estimates according to the following ranking: randomized controlled trial, regression discontinuity design, instrumental variables, difference-in-differences, and ordinary least squares. For a given methodology, in cases in which there was more than one specification and the study did not clearly state the preferred estimate, we used the specification with the most

controls. Last, if estimates were given for multiple independent samples, we computed a single observation-weighted average of the sample estimates and combined standard errors (42). Estimates were coded by the authors according to this protocol and independently verified by a second coder. Details on how estimates were coded are available in the supplementary materials (42).

Because some papers report estimates from different samples, we assembled a second database in which we extracted multiple estimates from a study when the estimates are from distinct samples. This database, which we denote the sample-level database, contains 46 estimates from the 34 studies. For each sample, we coded whether the job required abstract reasoning, the incentive structure of the job (fixed wages, individual piece rates, or team-based compensation), whether workers were substitutes or complements in the production process, and whether workers competed over scarce inputs to worker-level production. This sample-level database complements the study-level data set by allowing us to examine how worker spillover estimates vary by study characteristic because papers sometimes report estimates for different workplace conditions. Within the sample-level database, in 48% of samples, workers had fixed wages with no individual piece rate compensation; in 48% of samples, workers were paid with individual piece rates; and in 20% of samples, there was team-based compensation.

In 24% of samples, the task or occupation required abstract reasoning (Table 1).

Twenty-two studies in our study-level database relate a worker's productivity to the productivity of a co-worker or group of co-workers (a levels-levels specification). Because the dependent and independent variables of interest are in the same units, the estimated coefficient is a unitless measure that can be interpreted as an elasticity or a standardized coefficient. In seven studies, the dependent and independent variables are in natural log units. As long as mean productivity is similar between focal and peer workers, means of the dependent and independent variables are close, and the estimated coefficients have a similar interpretation as the levels-levels specification [this follows from $d\ln(y)/d\ln(x) \approx (dy/dx)(\bar{x}/\bar{y})$]. Excluding estimates from log specifications in our data set yields similar pooled estimates of $\hat{\gamma}$ as the main sample (table S2). In five studies, the dependent and independent productivity measures are in different units, in which case we standardized both variables with respect to the individual-level productivity distribution. Because of the presence of these five studies, the most precise interpretation of the mean of $\hat{\gamma}_i$ across studies (denoted $\hat{\gamma}$) is a standardized coefficient giving the standard deviation change in worker productivity from a 1 SD change (in the individual-level productivity distribution) of co-worker productivity. However, $\hat{\gamma}$ also has an

Table 1. Summary statistics. Shown are unweighted summary statistics for study-level and sample-level databases. Peer-effects estimates are denoted by $\hat{\gamma}$. “P value” corresponds to the P value of the estimated $\hat{\gamma}_i$ in the study. “Sample size” gives mean and standard deviation of sample sizes across papers. “Lab experiment” is a dummy variables for whether a study was classified as a laboratory experiment. “Published” indicates whether a study was published in a peer-reviewed journal. “Fixed wage” is a dummy variable for whether compensation had a flat or hourly pay component with no individual piece rate. “Individual piece rate” and “Group piece rate” are dummy variables for whether a portion of compensation was determined by individual or group output, respectively. “Complement” is a dummy for whether authors made specific reference to complementarities between workers in their joint production function. “Perfect substitute” is a dummy for whether workers in the sample being studied generated perfectly substitutable output in the production process. “Complex job” is a dummy for whether the job or task performed required abstract reasoning. “Rival” is a dummy for whether workers in the sample competed in the production process. “Observations” reports total number of estimates in the study-level and sample-level data sets, respectively. Dashes indicate that the variable was not coded in the database.

Variable	Study		Sample	
	Mean	SD	Mean	SD
$\hat{\gamma}$	0.134	0.203	0.140	0.216
P value	0.138	0.232	0.146	0.257
Sample size	468,375	2,207,142	155,511	344,550
Lab experiment	0.324	0.475	0.326	0.474
Published	0.500	0.508	0.543	0.504
Fixed wage	—	—	0.478	0.505
Individual piece rate	—	—	0.478	0.505
Group piece rate	—	—	0.196	0.401
Complement	—	—	0.022	0.147
Perfect substitute	—	—	0.565	0.501
Complex job	—	—	0.239	0.431
Rival	—	—	0.087	0.285
Observations		34		46

approximate elasticity interpretation: A 1% increase in average co-worker productivity increases focal worker productivity by $\hat{\gamma}$ percent. Excluding the studies with standardized variables and keeping estimates that have a pure elasticity interpretation yields an almost identical average estimate of $\hat{\gamma}$, as does converting the standardized measures into elasticities when possible and computing $\hat{\gamma}$ over all studies with a natural elasticity interpretation (table S2).

The average unweighted estimate of $\hat{\gamma}$ is 0.13 (SD = 0.20) (Table 1). In the study-level database, 56% of estimates are positive and statistically

significant at the 5% level, 2.9% of studies are negative and significant at the 5% level, and the remaining studies are insignificant at the 5% level (Fig. 1).

To aggregate estimates into a comprehensive summary measure, taking into account the sampling error of the estimates, we estimated a random-effects model that assumes that estimates are drawn from sampling distributions with possibly distinct means. The random-effects model assumes that each study's observed peer effects estimate is composed of three elements: the mean effect size γ , a study's divergence from the mean

effect λ_i , and an error term ϵ_i around that study's estimate. For study i , the observed peer effect estimate $\hat{\gamma}_i$ is given by

$$\hat{\gamma}_i = \gamma + \lambda_i + \epsilon_i$$

To estimate the summary effect $\hat{\gamma}$, study-level estimates are weighted by inverse variance weights

$$W_i = \frac{1}{SE_i^2 + \tau^2}$$

where SE_i^2 is the squared standard error of estimate i and τ^2 is the estimated between-study

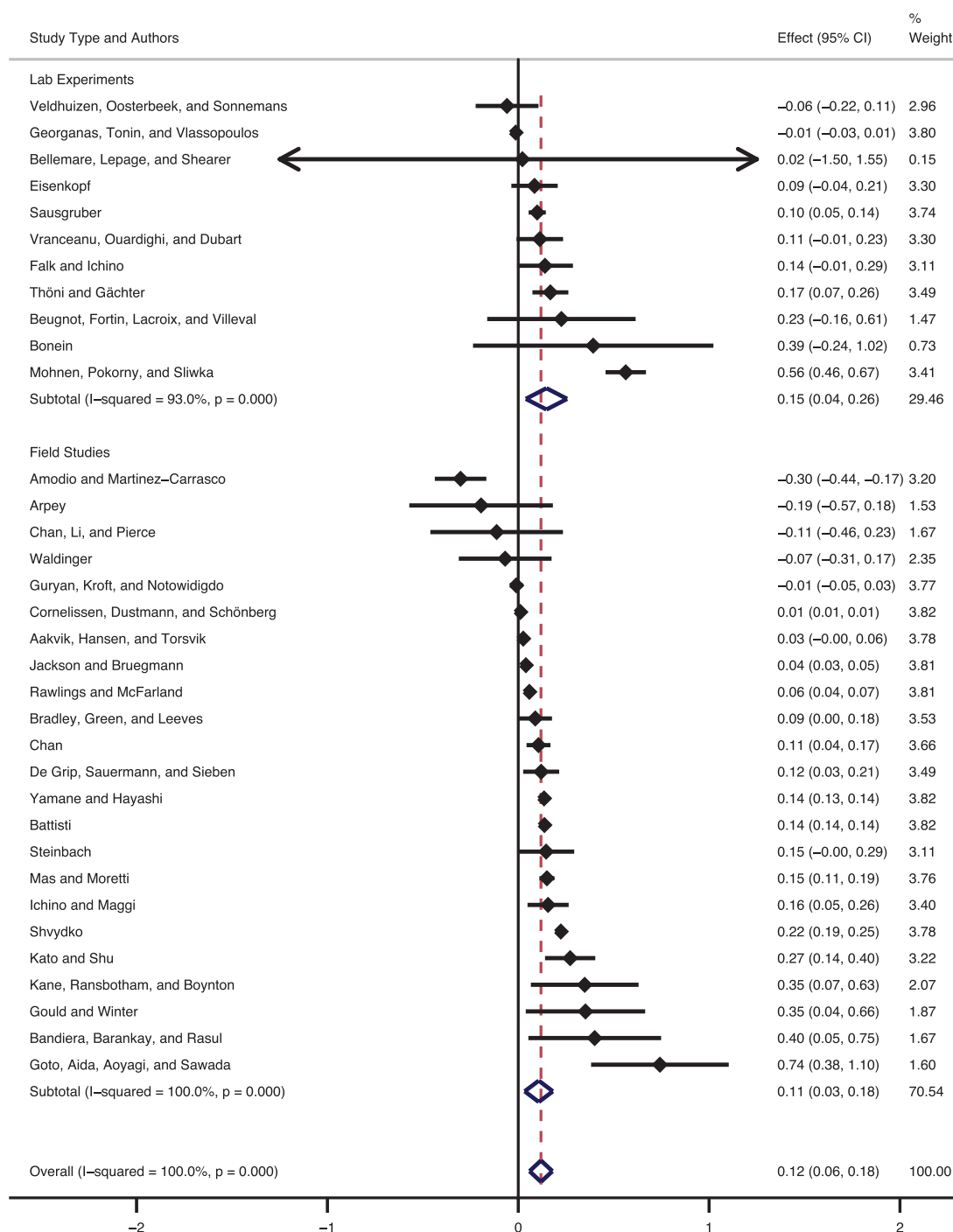


Fig. 1. All study-level estimates, summary effects by study type, and the overall summary effect. Solid black diamonds and lines show 95% confidence intervals around each study's estimated peer effect. The first two blue diamonds are centered around estimated summary peer effects by subgroup (laboratory versus field), and the dashed line and lowermost diamond are centered around the estimated overall summary effect. Widths of blue diamonds correspond to estimated 95% confidence intervals for summary effects. "% Weight" corresponds to the inverse-variance weight, where variance is computed as the reciprocal sum of each study's sample variance and the between-study variance: $W_i = (\sigma_i^2 + \tau^2)^{-1}$, normalized to sum to 100%.

Table 2. Summary estimates by paper type. Shown are the random-effects summary estimate of γ across laboratory and field studies. Standard errors are in parentheses. Observations are inverse variance–weighted, where variance is computed as the reciprocal sum of each study's sample variance and the between-study variance: $W_i = (\sigma_i^2 + \tau^2)^{-1}$. Standard error of the summary estimate is computed as the square root of the reciprocal sum of W_i . Final column reports P values for a comparison of means z test for equality in summary effect and τ^2 terms between laboratory and field studies. P value for τ^2 difference was computed using bootstrapped standard errors.

	Overall	Laboratory	Field	P value
Summary effect	0.120 (0.031)	0.148 (0.055)	0.107 (0.038)	0.545
τ^2	0.025	0.023	0.026	0.890
Observations	34	11	23	

Table 3. Laboratory-field comparisons controlling for sample characteristics. Shown are meta-regression coefficients for the sample-level data set with standard errors in parentheses. * $P < 0.10$, ** $P < 0.05$, *** $P < 0.01$. Dependent variable is study's reported worker peer effect estimate γ_i for sample i . "Laboratory experiment" is an indicator for whether or not an estimate was taken from a laboratory study. "Group piece rate" is a dummy variables for whether a portion of compensation was determined by group output. "Fixed wage" is a dummy variable for whether compensation had a flat or hourly pay component with no individual piece rate. "Published" indicates whether a study was published in a peer-reviewed journal. "Complex job" is a dummy for whether the job or task performed required abstract reasoning. "Perfect substitute" is a dummy for whether workers in the sample being studied generated perfectly substitutable output in the production process. "Complement" is a dummy for whether authors made specific reference to complementarities between workers in their joint production function. "Rival" is a dummy for whether workers in the sample competed in the production process. "GPR*Lab," "FW*Lab," and "PS*Lab" are interaction terms for "Laboratory experiment" with "Group piece rate," "Fixed wage," and "Perfect substitute," respectively. Estimates were obtained via variance-weighted least squares regression, where weights and standard errors are consistent with a random-effects meta-analysis model. Inverse-variance weights are computed as the reciprocal sum of each study's sample variance and the between-study variance: $W_i = (\sigma_i^2 + \tau^2)^{-1}$.

	(1)	(2)	(3)	(4)
Laboratory experiment	0.052 (0.059)	0.016 (0.062)	0.029 (0.059)	−0.050 (0.102)
Group piece rate		0.149** (0.073)	0.162** (0.070)	0.174* (0.097)
Fixed wage			0.099* (0.049)	0.046 (0.059)
Published		0.054 (0.055)	0.044 (0.053)	0.026 (0.056)
Complex job		−0.087 (0.067)	−0.085 (0.064)	−0.090 (0.065)
Perfect substitute		−0.106* (0.053)	−0.108** (0.050)	−0.113* (0.059)
Complement		−0.135 (0.171)	−0.082 (0.164)	−0.122 (0.177)
Rival		−0.188** (0.089)	−0.155* (0.086)	−0.182* (0.092)
GPR*Lab				0.002 (0.146)
FW*Lab				0.181 (0.114)
PS*Lab				−0.023 (0.130)
Constant	0.095*** (0.032)	0.152*** (0.052)	0.096* (0.056)	0.143** (0.064)
Observations	46	46	46	46

variance of estimates. The between-study variance is estimated with the empirical Bayes method (47), which has been shown to be more accurate than alternatives when the heterogeneity of estimates is large (48). The standard error of $\hat{\gamma}$ is given by

$$SE(\hat{\gamma}) = \frac{1}{\sqrt{\sum_i W_i}}$$

In the random-effects model, the majority of papers receive comparable weight, with a small handful of papers with imprecise estimates receiving little weight (Fig. 1). The average value of $\hat{\gamma}_i$ in the pooled sample is $\hat{\gamma} = 0.12$ ($SE = 0.03$, $n = 34$ studies) with an estimated between-study variance $\tau^2 = 0.025$ (Table 2). The I^2 statistic, which describes the percentage of total variation across studies that is due to heterogeneity rather than chance (42), is almost 100% for the pooled sample. The average value of $\hat{\gamma}_i$ for laboratory and field samples respectively is 0.148 ($SE = 0.055$, $n_{\text{lab}} = 11$ studies) and 0.107 ($SE = 0.038$, $n_{\text{field}} = 23$ studies). The random-effect average pooled estimates for both the laboratory and field estimates are statistically distinguishable from zero at conventional levels. We cannot reject equality of the means of γ in the laboratory and field samples ($P = 0.55$). The between-study variances are also similar in the laboratory and field samples: The between-study variability of estimates, τ^2 , is estimated as 0.023 in the laboratory sample and 0.026 in the field sample, and we cannot reject equality of these parameters using bootstrapped standard errors ($P = 0.89$).

Studies differ in compensation schemes, production processes, and task complexity. These features of the studies may influence the extent of peer effects. The small mean difference between estimates in the laboratory and field samples is stable after controlling for study and workplace characteristics in a regression framework. We used the sample-level data set to estimate the following equation using a modified variance-weighted least-squares regression:

$$\hat{\gamma}_i = \alpha_i + \beta \text{lab}_i + \delta X_i + \epsilon_i$$

where lab_i is an indicator for whether the estimate was taken from a laboratory experiment, X_i is a vector of variables controlling for study and workplace characteristics, ϵ_i is the error term, and each observation is weighted by its inverse variance W_i (Table 3). Without controls, the estimated laboratory experiment indicator coefficient ($\hat{\beta}$) is similar in magnitude to the value found in the study-level data set ($\hat{\beta} = 0.05$, $P = 0.38$, $n = 46$ samples). The estimates attenuated when we added workplace and paper characteristics, including variables for worker compensation incentives (group piece-rate indicator and fixed-wage indicator), publication status, an indicator for whether the occupation requires abstract reasoning, and an indicator for whether workers in the study are rivals ($\hat{\beta} = 0.029$, $P = 0.62$, $n = 46$ samples).

The coefficients on the workplace characteristics in the regression setting are worth

additional discussion. As has been long noted in the literature on organizations (49), workplaces with team-based production and fixed wages are susceptible to the free-rider problem because effort is a public good when there is imperfect monitoring. Assuming workers only have self-interested motives, introducing a more productive co-worker in such a workplace will lead other workers to be less productive because their colleague is taking on a greater share of the workload. However, the free-rider problem can be overcome with mutual monitoring or the threat of social sanctions. In this case, the presence of a more productive co-worker might lead other workers to increase their effort because of these considerations. The coefficients on the group piece-rate and fixed-wage coefficients are both positive and statistically significant, suggesting that positive co-worker productivity spillovers are particularly important in these settings. This finding is evidence that positive co-worker peer effects, possibly because of the threat of social sanctions, help mitigate the free-rider problem. The negative and significant coefficient on substitutes indicates that production processes in which workers are perfect substitutes have less pronounced peer-productivity spillovers. We tested whether these conclusions differ between laboratory and field studies. Estimating a model with interactions of the laboratory indicator with indicators for group piece-rate, fixed wages, and perfect substitutability (variables for which there is sufficient overlap between laboratory and field studies), we found coefficients on interaction terms for group piece rate and substitutability that are close to zero and insignificant, whereas the coefficient on the fixed-wage and laboratory interaction is positive but not statistically significant because of a large standard error (Table 3, column 4). The estimates on the interaction terms imply that laboratory and field studies yield similar relationships between workplace characteristics and the magnitude of peer effects for two of the three characteristics we examined (and inconclusive for the third), with the caveat that these estimates are somewhat imprecise.

We conclude that for estimation of γ , laboratory studies generalize quantitatively. This is a surprising finding because even proponents of laboratory experiments have argued that laboratory experiments may only generalize qualitatively (7), and it suggests that laboratory experiments have more external validity than previously recognized. One caveat is that there is between-study dispersion reflecting unobserved heterogeneity in worker peer effects estimates both for field and laboratory studies, so that any individual study differs from the mean of the summary $\hat{\gamma}$ estimate. However, we also found that between-study standard deviations are comparable in the laboratory and field samples, as are the prediction intervals. Consequently, laboratory experiments provide a representative depiction of the overall distribution of γ values.

An additional question is why estimation of productivity spillovers translates well in the laboratory. The findings suggest that it is possible to

simulate realistic work environments in the laboratory, particularly experiments with real-effort tasks. However, there are aspects of real workplaces that are missing in even the most complex laboratory studies. In laboratory studies, subjects are usually aware that they are being observed, and it is known that in a number of environments, there are effects of social facilitation on performance that are mediated by whether the task requires performance of learned skills or learning new skills (50). It is also not possible to simulate long-term employment relationships in the laboratory, a feature of many real workplaces. Our results indicate that these special features of the laboratory, among others, may not be as important for estimating productivity spillovers as the ones that are modeled.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/350/6260/545/suppl/DC1
Materials and Methods
Tables S1 and S2
Reference (51)

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CHRONIC ITCH

Gate control of mechanical itch by a subpopulation of spinal cord interneurons

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Light mechanical stimulation of hairy skin can induce a form of itch known as mechanical itch. This itch sensation is normally suppressed by inputs from mechanoreceptors; however, in many forms of chronic itch, including alopecia, this gating mechanism is lost. Here we demonstrate that a population of spinal inhibitory interneurons that are defined by the expression of neuropeptide Y::Cre (NPY::Cre) act to gate mechanical itch. Mice in which dorsal NPY::Cre-derived neurons are selectively ablated or silenced develop mechanical itch without an increase in sensitivity to chemical itch or pain. This chronic itch state is histamine-independent and is transmitted independently of neurons that express the gastrin-releasing peptide receptor. Thus, our studies reveal a dedicated spinal cord inhibitory pathway that gates the transmission of mechanical itch.

The sensation of itch elicits stereotypical scratching behaviors that are an important protective response to cutaneous irritants and parasites. Animals appear to have evolved two forms of itch: (i) chemical itch, which is activated by chemical mediators such as histamines and proteases (1–6) and can be effectively gated by noxious painful stimuli (7), and (ii) mechanical itch, which is evoked by light tac-

tile stimuli, such as when insects or parasites come in contact with the skin. In humans, this latter pathway can be activated by vibrating the fine vellous hair (8). Itching is also frequently evoked by light mechanical stimuli in patients suffering from chronic itch (9, 10).

Although progress has been made toward identifying the spinal inhibitory neurons that gate chemical itch (11, 12), little is known about the

spinal pathways gating mechanical itch. The dorsal horn of the spinal cord contains multiple inhibitory interneuron (IN) populations including cells that express neuropeptide Y (NPY) (13, 14). These cells are distinct from those that express dynorphin, galanin, neuronal nitric oxide synthase (nNOS), and parvalbumin (15, 16). When NPY::Cre transgenic mice [Gene Expression Nervous System Atlas (GENSAT), RH26 cell line (see supplementary materials and methods)] were crossed with *R26^{LSL-tdTomato}* (*ai14*) reporter mice to trace the provenance of the dorsal horn INs expressing NPY, NPY::Cre-derived INs were localized in laminae III and IV (70.4 ± 0.3%) and, to a lesser extent, in laminae I and II (29.6 ± 0.3%) (Fig. 1, A and B). The number of NPY⁺/tdTomato⁺ INs decreases postnatally, with only 35% of the tdTomato cells expressing NPY at postnatal day 30 (P30) (Fig. 1C). NPY::Cre thus captures two populations of NPY-expressing neurons: one that transiently expresses NPY during late embryonic and early neonatal development

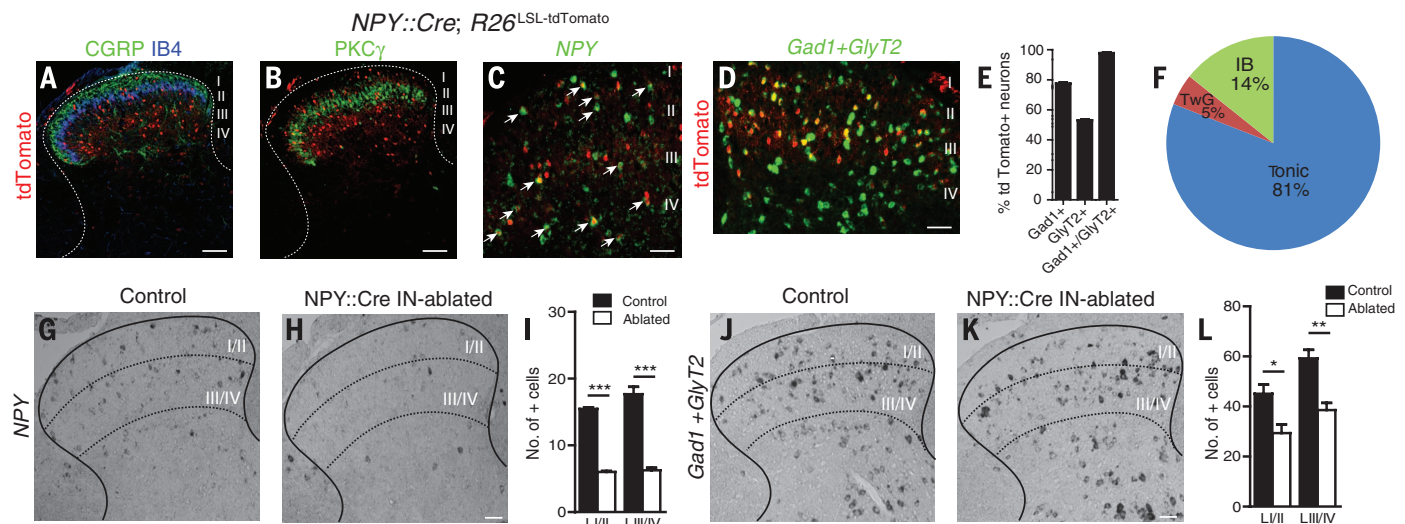


Fig. 1. NPY::Cre delineates a population of inhibitory neurons in the dorsal spinal cord. (A and B) Sections through the lumbar dorsal spinal cord of a P30 NPY::Cre; *R26^{LSL-tdTomato}* mouse stained with calcitonin gene-related peptide (CGRP) and IB4 (A) and protein kinase Cγ (PKCγ) (B). tdTomato⁺ fluorescence was visualized without staining. Roman numerals denote laminae. (C) A section through the lumbar dorsal spinal cord of a P30 NPY::Cre; *R26^{LSL-tdTomato}* mouse, comparing expression of tdTomato reporter (red) and NPY (green, in situ). Arrows indicate double-labeled cells. (D) A section through the lumbar dorsal horn of a P30 NPY::Cre; *R26^{LSL-tdTomato}*, showing coexpression of tdTomato with the inhibitory markers *Gad1* and *GlyT2*. (E) Quantification of coexpression of NPY-tdTomato⁺ with *Gad1*-GFP, *GlyT2*-GFP, and *Gad1*⁺/*GlyT2* in situ hybridization. (F) Firing properties of NPY::Cre INs. The majority of NPY-tdTomato⁺ INs (34 of 42 cells) display a tonic firing pattern upon current injection. IB, intermittent bursting; TwG, tonic firing with gap. (G to L) In situ analysis and quantification of NPY [(G) to (I)] and *Gad1*/*GlyT2* [(J) to (L)] expression in the lumbar dorsal spinal cord of P60 control and NPY::Cre IN-ablated mice. NPY⁺ cell numbers were reduced by 59.0 and 69.5% in laminae I and II and laminae III and IV, respectively [(I) ****P* < 0.001]. *Gad1*⁺ and *GlyT2*⁺ cell numbers were reduced by 34.7% in laminae I and II [(L) **P* < 0.05] and 34.9% in laminae III and IV [(L) ***P* < 0.01]. *P* values were calculated using the Student's unpaired *t* test. Scale bars: 50 μm [(C), (D), (H), and (K)]; 200 μm [(A) and (B)]. Error bars indicate SEM [(I) and (L)].

and another that shows persistent expression into adulthood.

More than 98% of the NPY::Cre-tdTomato⁺ cells expressed glutamic acid decarboxylase 1 (Gad1) and/or the glycine transporter 2 (GlyT2⁺) (Fig. 1, D and E). After current injection, the majority of cells (34 of 42) displayed a tonic firing pattern (Fig. 1F) that is characteristic of many dorsal horn inhibitory INs (17). These NPY::Cre INs make up 31 and 45% of the inhibitory Gad1⁺ or GlyT2⁺ INs in laminae I and II, and in laminae III and IV, respectively. Very few of the NPY::Cre INs cells expressed nNOS (4.9 ± 0.6%), galanin (5.0 ± 1.1%), dynorphin (8.2 ± 1.6%), or parvalbumin (5.9 ± 1.7%), indicating that they constitute a distinct population of dorsal horn inhibitory INs.

An intersectional genetic strategy that restricts diphtheria toxin receptor (DTR) expression to NPY::Cre-derived INs in the dorsal spinal cord and medulla (18) was then used to determine the contribution the NPY::Cre INs make to gating cutaneous sensory stimuli. Injecting NPY::Cre; *Lbx1*^{FlpO}; *Tau*^{ds-DTR} mice with diphtheria toxin (DTX) markedly reduced the number of NPY::Cre-tdTomato INs in the dorsal spinal cord (fig. S1, A to C). This cell loss was restricted to inhib-

itory INs that express NPY, Gad1 and/or GlyT2 (Fig. 1, G to L, and fig. S1, G to I), and Pax2 (fig. S1, D to F). Neighboring dorsal inhibitory IN subtypes expressing nNOS, dynorphin, and parvalbumin (fig. S1, J to R) were spared, as were dorsal excitatory IN subtypes (fig. S2, A to L). There was no noticeable change in the central projections of sensory afferent nerve fibers or in the distribution of NPY::Cre-derived neurons in other regions of the central nervous system (fig. S3, A and B).

Two weeks after injection with DTX, the NPY::Cre IN-ablated mice began to display spontaneous scratching, followed by the appearance of skin lesions (Fig. 2, A and B). This scratching was not related to chemical itch, as injection of chemical pruritogens (compound 48/80 and chloroquine) into the nape region of NPY::Cre IN-ablated mice before the onset of spontaneous scratching revealed no difference in the level or intensity of scratching (Fig. 2, C and D). Following a modified protocol for analyzing allodynia in mice in which von Frey hairs were used to deliver graded mechanical forces to the nape of the neck (19) (Fig. 2E), we observed a significant increase in evoked hindlimb scratching in NPY::Cre IN-ablated mice with low-force (0.02 to 0.4 g)

von Frey hairs as compared with control mice (Fig. 2F). In contrast, high-threshold mechanical stimuli (0.6 to 1 g) did not induce pronounced scratching. Additional behavioral tests revealed no marked differences between control and NPY::Cre IN-ablated mice with regard to their responsiveness to noxious mechanical and thermal stimuli (fig. S4A). Acute chemical pain sensitivity was also normal: Injection of capsaicin into the cheek of control and NPY::Cre IN-ablated mice produced similar levels of pain-indicating wiping, with little or no itch-indicating scratching (20) (fig. S4B).

To rule out the possibility that the increased scratching in NPY::Cre IN-ablated mice arises from secondary changes to the spinal circuitry after neuronal ablation, we used an intersectional genetic strategy—which involves mice that carry a conditional double-stop allele encoding the inhibitory hM4D DREADD receptor (21) (*R26*^{ds-hM4D-tdTomato}) (fig. S5, A and B)—to acutely silence the NPY::Cre-derived INs (Fig. 2G). Activation of hM4D with clozapine *N*-oxide (CNO) precipitated a mechanical itch phenotype that closely resembled the itching behavior observed after NPY::Cre IN ablation (Fig. 2H). Forty min after CNO injection, low-threshold mechanical

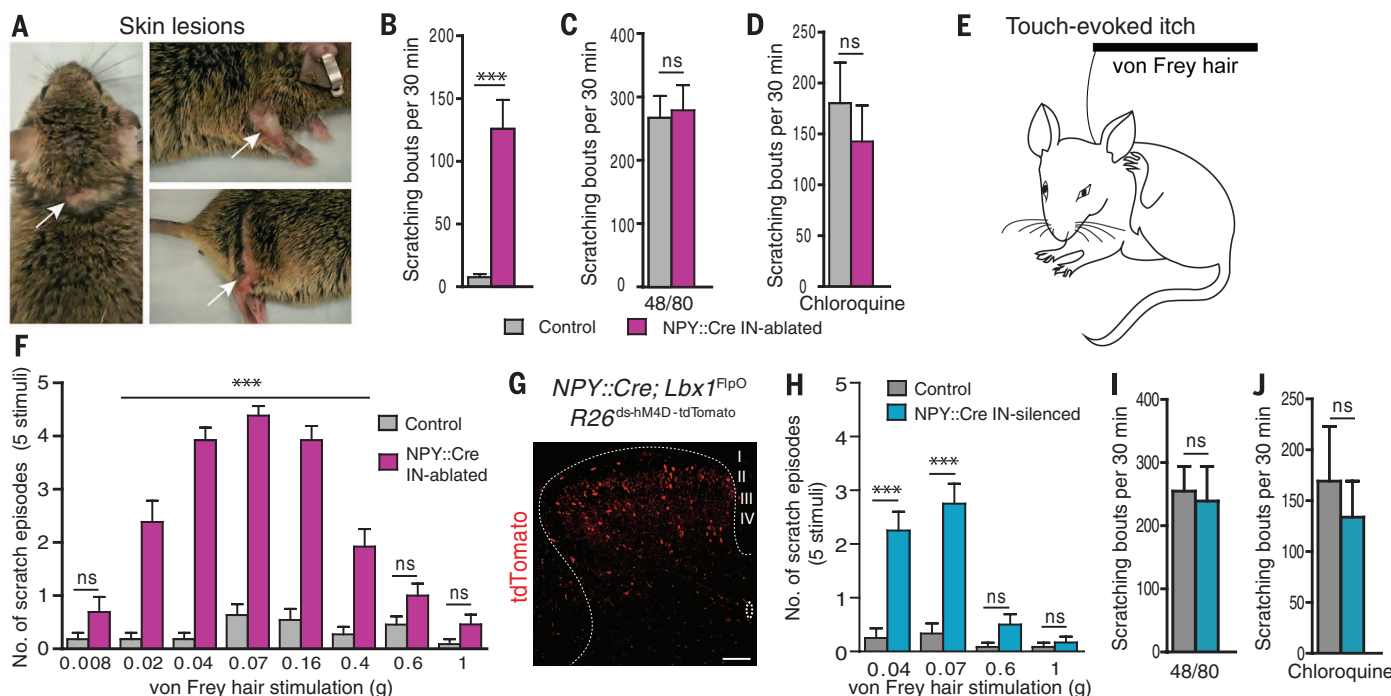


Fig. 2. Ablation or silencing of NPY::Cre INs induces touch-evoked itch.

(A) Skin lesions on the neck and body of NPY::Cre IN-ablated mice 2 weeks after injection with DTX. (B) Number of spontaneous scratch events over a 30-min period (control: 75 ± 2.6; NPY::Cre IN-ablated: 125.8 ± 23.2; *n* = 10 mice; ****P* < 0.001). (C and D) Equivalent responses to chemical-evoked itch are seen in control and NPY::Cre IN-ablated mice 7 days after DTX injection and before development of the spontaneous scratch phenotype. (C) Compound 48/80 (control: 266.8 ± 34.9; NPY::Cre IN-ablated: 279 ± 39.6; *n* = 6 mice; *P* = 0.82). (D) Chloroquine (control: 180.1 ± 39.8, *n* = 7 mice; NPY::Cre IN-ablated: 142.5 ± 35.3, *n* = 6 mice; *P* = 0.50). (E) Schematic showing the touch-evoked itch (allodynia) test. (F) Increase in the number of scratch events after applying von Frey hairs to the shaved nape of the neck (control: *n* = 11 mice; NPY::

Cre IN-ablated: *n* = 13 mice; ****P* < 0.001). (G) tdTomato reporter expression in a section through the lumbar dorsal spinal cord of NPY::Cre; *Lbx1*^{FlpO}; *R26*^{ds-hM4D-tdTomato} mice. Scale bar, 200 μm. (H) An increase in the number of scratches associated with low-intensity force (0.04 and 0.07 g), but not high-intensity force (0.6 and 1 g), is seen in NPY::Cre IN-silenced mice compared with control mice (control: *n* = 12 mice; NPY::Cre IN-silenced: *n* = 12 mice; ****P* < 0.001). (I and J) Equivalent responses to chemical-evoked itch are seen in control and NPY::Cre IN-silenced mice. (I) Compound 48/80 (control: 254.8 ± 39.03; NPY::Cre IN-silenced: 239 ± 54.78; *n* = 6 mice; *P* = 0.82). (J) Chloroquine (control: 169.3 ± 53.6, *n* = 6 mice; NPY::Cre IN-silenced: 133.9 ± 35.49, *n* = 7 mice; *P* = 0.58). ns, no significant difference. *P* values were calculated using the Student's unpaired *t* test. Error bars indicate SEM [(B) to (D), (F), and (H) to (J)].

stimuli (0.04 and 0.07 g) produced robust scratching, whereas high-threshold mechanical stimuli (0.6 and 1 g), which typically produce pain, did not. Silencing the NPY::Cre INs did not increase scratching behavior after exposure to compound 48/80 and chloroquine (Fig. 2, I and J). Responses to von Frey hairs, brush stroke, and the Hargreaves test were also unchanged (fig. S5C), and there was no increase in mechanical allodynia after injection with Freund's complete adjuvant (fig. S5, D and E), which indicates that NPY::Cre-derived INs primarily inhibit mechanical itch.

Dorsal horn neurons that express the gastrin-releasing peptide receptor (GRPR) are required for itch transduction by a variety of chemical pruritogens (22, 23). To address whether the mechanical itch pathway gated by the NPY::Cre INs differs from this chemical itch pathway, chemical itch was blocked either pharmacologically or by ablating the GRPR neurons in the dorsal horn. As compared with saline-treated controls, NPY::Cre IN-ablated mice treated with a H1/H4 histamine receptor antagonist displayed no reduction in the number of scratch events after mechanical stimulation on the nape of the neck (Fig. 3A), despite the antagonist being highly effective in reducing compound 48/80-induced scratching (Fig. 3B). Although intrathecal injection of a GRPR antagonist or ablation of GRPR-expressing neurons with conjugated bombesin-saporin (fig. S6) was effective in blocking chloroquine-evoked itch (22) (Fig. 3, D and F), these manipulations failed to blunt scratching in response to mechanical stimulation in NPY::Cre IN-ablated mice (Fig. 3,

C and E). Mechanical itch gated by NPY::Cre INs is therefore independent of the histaminergic and GRP-GRPR itch pathways described to date (1–7).

To assess whether the NPY::Cre INs contribute to the tactile inhibition of itch, we asked if the NPY::Cre INs are innervated by cutaneous low-threshold mechanoreceptors (LTMs). In *Pitx2-EGFP* mice (GENSAT; EGFP, enhanced green fluorescent protein), myelinated hair follicle afferent fibers that selectively express GFP (fig. S7, A to C) form multiple contacts on the cell bodies and dendrites of NPY::Cre INs (Fig. 4, A and B). When cholera toxin B (CTB) was injected into the hairy skin (fig. S7, D to G) (24), presumptive CTB⁺/vGluT1⁺ A β - and A δ -LTM synaptic boutons and putative CTB⁺/vGluT1⁺ C-LTM synaptic contacts were detected on NPY::Cre-tdTomato⁺ INs in laminae III and IV (Fig. 4C and fig. S7Ga, arrows) and lamina II (Fig. 4C and fig. S7Gb), respectively. The synaptic nature of these contacts was confirmed by single-synapse transsynaptic rabies tracing (Fig. 4, D to G, and fig. S7, H to J) and whole-cell recordings from NPY::Cre-tdTomato neurons (Fig. 4H). Our demonstration that the NPY::Cre INs receive LTM inputs, coupled with evidence from humans that mechanical itch is gated by LTMs (8), suggests that NPY::Cre INs mediate the tactile inhibition of mechanical itch.

We next examined how neurons in laminae I to III respond to innocuous touch (brush stroke) (Fig. 4, I and J) and painful stimuli (pinch) (fig. S8, B and C) after NPY::Cre IN ablation. Neurons

with hairy-skin receptive fields displayed a significant increase in afterdischarge spike number in NPY::Cre IN-ablated mice compared with control mice (Fig. 4I). This occurred in the absence of any concomitant increase in spontaneous activity (fig. S8A). In contrast, afterdischarge activity was unchanged following brush stroke in neurons with glabrous-skin receptive fields (Fig. 4J) or noxious stimulation (pinch) of both hairy and glabrous skin (fig. S8, B and C). Therefore, NPY::Cre INs have a specific role in gating innocuous mechanosensory inputs from hairy skin. This finding is consistent with our observation that scratching and skin lesions are restricted to hairy sites in NPY::Cre IN-ablated mice (Fig. 2A), and sensitivity to noxious or mechanical stimulation on the glabrous skin is unchanged (fig. S4).

Our findings reveal that inhibitory spinal INs marked by the expression of NPY::Cre selectively gate low-threshold mechanical itch. In contrast, *Bhlhb5* conditional knockout mice show increased sensitivity to chemical itch (11). NPY expression is not affected in the *Bhlhb5* mutant cord (17), and several *Bhlhb5*-dependent inhibitory IN subtypes are spared after NPY::Cre IN ablation (fig. S1). This suggests that NPY::Cre-derived and *Bhlhb5*-dependent inhibitory INs are required to gate mechanical and chemical itch pathways, respectively (summarized in Fig. 4K). The loss of NPY::Cre INs (fig. S4) or *Bhlhb5*-dependent inhibitory INs (11) does not affect mechanical pain, which is gated by dynorphin-expressing inhibitory INs (18). Therefore, inhibitory INs in the dorsal horn appear to be organized into discrete

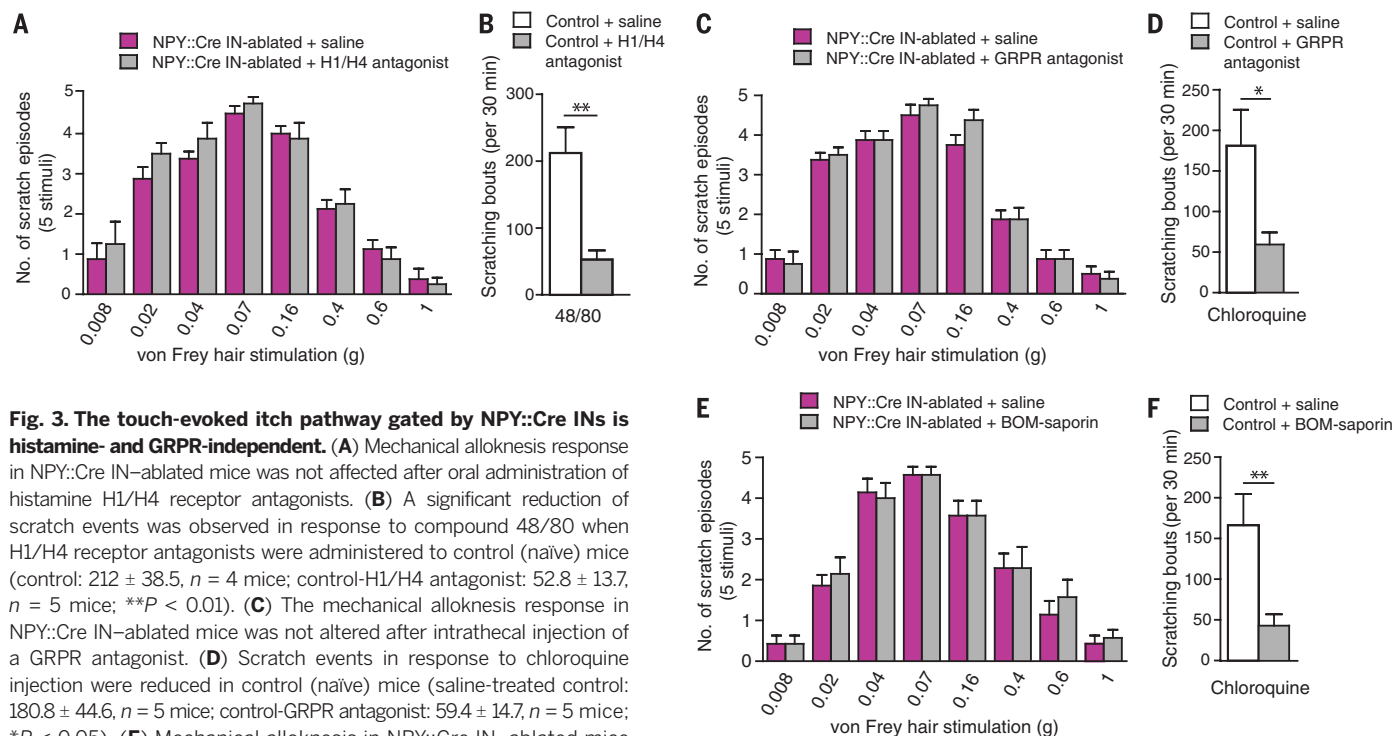


Fig. 3. The touch-evoked itch pathway gated by NPY::Cre INs is histamine- and GRPR-independent. (A) Mechanical allodynia response in NPY::Cre IN-ablated mice was not affected after oral administration of histamine H1/H4 receptor antagonists. (B) A significant reduction of scratch events was observed in response to compound 48/80 when H1/H4 receptor antagonists were administered to control (naïve) mice

(control: 212 ± 38.5 , $n = 4$ mice; control-H1/H4 antagonist: 52.8 ± 13.7 , $n = 5$ mice; $**P < 0.01$). (C) The mechanical allodynia response in NPY::Cre IN-ablated mice was not altered after intrathecal injection of a GRPR antagonist. (D) Scratch events in response to chloroquine injection were reduced in control (naïve) mice (saline-treated control: 180.8 ± 44.6 , $n = 5$ mice; control-GRPR antagonist: 59.4 ± 14.7 , $n = 5$ mice; $*P < 0.05$). (E) Mechanical allodynia in NPY::Cre IN-ablated mice was not affected 2 weeks after ablation of GRPR⁺ INs in the spinal cord.

(F) Ablation of the GRPR⁺ INs in control (naïve) mice caused a significant reduction of scratch events induced by chloroquine injection [saline-treated control: 166.1 ± 38.5 , $n = 7$ mice; bombesin-saporin (BOM-saporin)-treated control: 42.9 ± 13.9 , $n = 7$ mice; $**P < 0.01$]. P values were calculated using the Student's unpaired t test. Error bars indicate SEM.

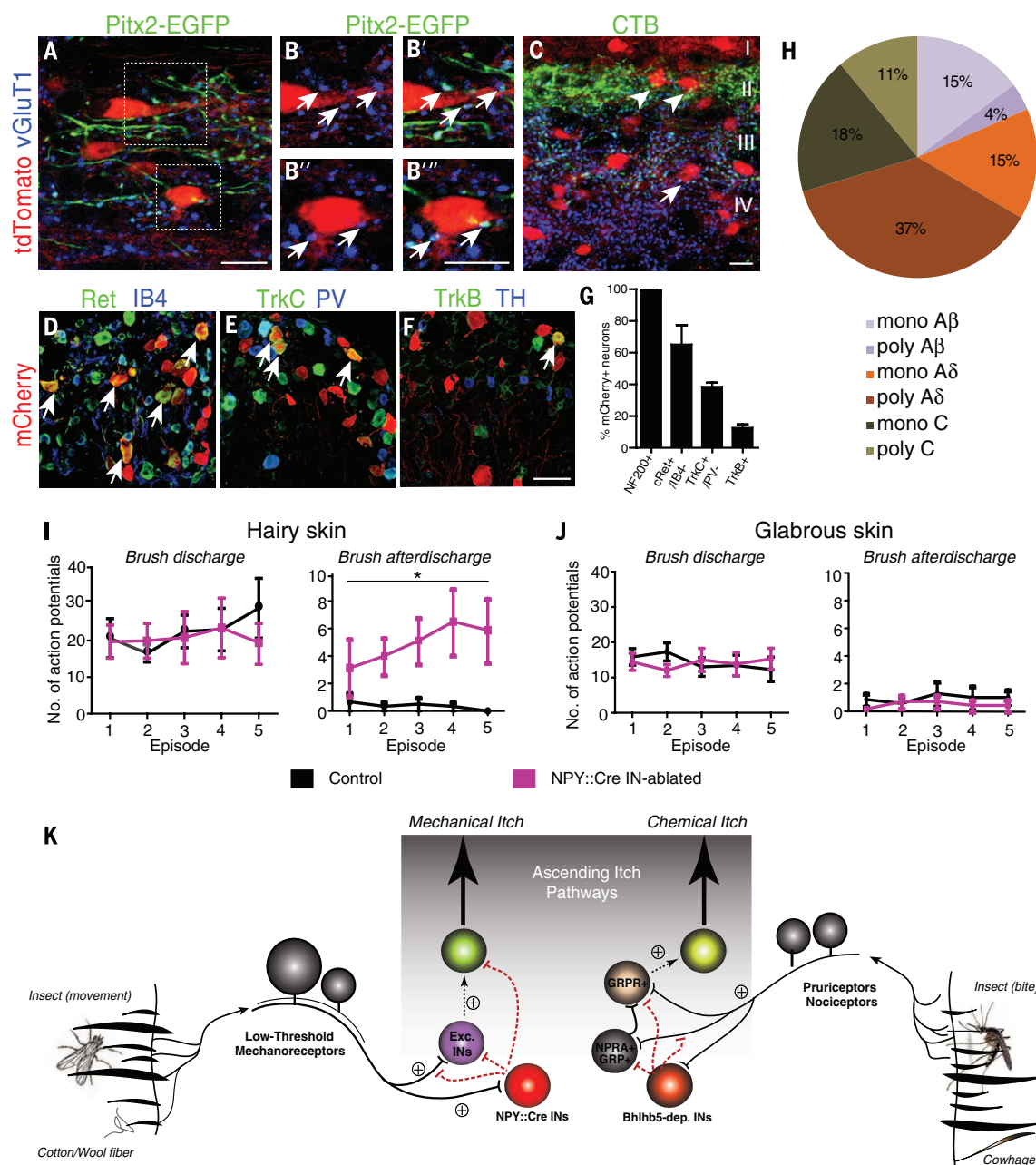


Fig. 4. NPY::Cre INs form a feedforward inhibitory pathway from hairy skin to suppress mechanical itch. (A to B'') Sections from the lumbar dorsal spinal cord of NPY::Cre; *R26^{LSL-tdTomato}*; *Pitx2-EGFP* mice, showing *Pitx2-EGFP*⁺ LTM synaptic terminal afferent fibers (GFP⁺/vGluT1⁺) in close apposition to NPY::Cre-TdTomato⁺ INs. Higher-magnification images of the boxed areas in (A) are shown in (B) to (B''). Arrows indicate synaptic contacts. (C) Sections from lumbar dorsal spinal cord of NPY::Cre; *R26^{LSL-tdTomato}* mice 3 days after injection of CTB in hairy skin stained with the indicated markers. Arrows indicate myelinated contacts; arrowheads denote unmyelinated contacts. (D to G) Transynaptic labeling of LTMs after selective infection of NPY::Cre INs with pseudotyped EnvA-mCherry rabies virus. Shown are representative sections through the dorsal root ganglion of a P13 NPY::Cre; *Lbx1^{FlpO}*; *R26^{ds-rtTA}* mouse stained with the indicated markers of LTM subtypes [(D) to (F)]. Arrows indicate double labeled neurons. (G) Quantification of sensory neuronal markers in relation to the total number of mCherry⁺ neurons. Data: mean $\pm$ SEM; $n = 3$ mice. (H) Classification of potentials in NPY::Cre INs induced by dorsal root stimulation. Of the 27 recorded cells, 3 cells with a monosynaptic A β input displayed a second monosynaptic A δ (1 cell) and polysynaptic C (2 cells) input. (I and J) In vivo extracellular recordings from lumbar

dorsal spinal cord neurons in response to mechanical stimulation of hairy and glabrous skin. (I) Increase in the mean of afterdischarge firing of neurons with hairy-skin receptive fields in NPY::Cre IN-ablated mice compared with control mice (control: 0.4 ± 0.3 spikes/s, $n = 7$ cells; NPY::Cre IN-ablated: 4.9 ± 1.4 spikes/s, $n = 7$ cells; two-way analysis of variance, $*P = 0.03$). The number of spikes fired during active brushing was unchanged (control: 22.3 ± 4.2 spikes/s, $n = 7$ cells; NPY::Cre IN-ablated: 20.1 ± 5.7 spikes/s, $n = 7$ cells). (J) Afterdischarge firing of neurons with glabrous-skin receptive fields in NPY::Cre IN-ablated compared with control mice (control: 0.9 ± 0.3 spikes/s, $n = 5$ cells; NPY::Cre IN-ablated: 0.5 ± 0.2 spikes/s, $n = 7$ cells). The number of spikes fired during active brushing was unchanged (control: 14.1 ± 2.4 spikes/s, $n = 5$ cells; NPY::Cre IN-ablated: 15.3 ± 2.8 spikes/s, $n = 7$ cells). (K) Model for the mechanical itch pathway: Light-touch stimuli on hairy skin stimulates LTMs to evoke mechanical itch. This itch pathway is gated by other LTMs via their activation of inhibitory NPY::Cre INs. The mechanical itch circuit is independent of the chemical itch pathways transduced by natriuretic peptide receptor A, GRP, and GRPR (26), which are gated by inhibitory Bhlhb5-dependent INs. Scale bars: 10 μ m [(A) to (C)]; 50 μ m (F). Error bars indicate SEM [(G), (I), and (J)].

functional modules that gate different streams of somatosensory information.

In identifying a previously uncharacterized gate for low-threshold mechanical itch, this study highlights a largely overlooked driver of chronic itch—namely, the light-touch pathway that is insensitive to antihistamine or anti-GRPR drugs (8). Human patients with chronic itch (trichoknesis) (25) display a phenotype similar to that seen in the NPY::Cre IN-ablated mice. In both instances, itch sensitivity is restricted to the hairy skin. Our finding that the NPY::Cre-derived INs are innervated by tactile inputs from hairy-skin LTMs (Fig. 4) suggests that the NPY::Cre INs are key components of a spinal inhibitory circuit by which hairy-skin LTMs gate mechanical itch. We propose that the NPY::Cre INs function as rheostat for low-threshold tactile stimuli, suppressing itch and preventing excessive scratching.

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BRAIN DEVELOPMENT

A GABAergic projection from the zona incerta to cortex promotes cortical neuron development

Jiadong Chen* and Arnold R. Kriegstein*

γ -Aminobutyric acid (GABA) is the major inhibitory transmitter in the mature brain but is excitatory in the developing cortex. We found that mouse zona incerta (ZI) projection neurons form a GABAergic axon plexus in neonatal cortical layer 1, making synapses with neurons in both deep and superficial layers. A similar depolarizing GABAergic plexus exists in the developing human cortex. Selectively silencing mouse ZI GABAergic neurons at birth decreased synaptic activity and apical dendritic complexity of cortical neurons. The ZI GABAergic projection becomes inhibitory with maturation and can block epileptiform activity in the adult brain. These data reveal an early-developing GABAergic projection from the ZI to cortical layer 1 that is essential for proper development of cortical neurons and balances excitation with inhibition in the adult cortex.

During embryonic development, neural activity (1, 2) influences proliferation, migration, and differentiation, as well as circuit refinement (3–5). In immature brains, the neurotransmitter GABA has excitatory effects due to high intracellular chloride (6, 7), contrary to its inhibitory effects in adult brains. GABA in the immature neocortex comes from local interneurons and axonal projections from other brain regions (8–12). The neonatal rodent brain has an excitatory GABAergic plexus projecting widely within cortical layer 1 (13, 14). Here, we show that the zona incerta (ZI) generates the neurons of this GABAergic plexus.

We mapped the ZI pathway in transgenic mice by manipulating channelrhodopsin-2 (ChR2) expression in somatostatin (SST)-expressing neurons in the ZI of neonatal mice (postnatal day P0–P1) (15, 16). Labeling was restricted to ZI GABAergic (Fig. 1A), SST⁺ (Fig. 1C) neurons 1 week after virus injection, and we observed EYFP⁺ ZI axonal projections widely distributed in layer 1 of somatosensory and motor cortex (Fig. 1, A and B, and fig. S1). At P7, ChR2 was reliably expressed in ZI neurons, and blue light stimulation induced firing of EYFP⁺ ZI neurons (Fig. 1, D to F). We filled layer 5 cortical neurons with neurobiotin in acute slices of somatosensory and motor cortex and coimmunostained with the cortical layer 5 marker Ctip2 (Fig. 1I, 16/16 neurons). The apical dendrites of these pyramidal neurons contacted layer 1 EYFP⁺ axons (Fig. 1G) and colocalized with the GABAergic presynaptic marker vGat (vesicular GABA transporter) (Fig. 1H).

Blue light stimulation of layer 1 evoked synaptic responses in layer 4 and layer 5 pyramidal neurons. The light-evoked responses were not sensitive to α -amino-3-hydroxy-5-methyl-4-isoxazole propi-

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Morphologically, the human brain has abundant GABAergic synapses in cortical layer 1 as early as gestational week (GW) 12 (17, 18). In the second trimester, subplate neurons show spontaneous firing and synaptic activity (19, 20). Examining the expression and distribution of GABAergic axons in human cortex at GW 24, we found that both the axon marker neurofilament-2H3 and the GABAergic presynaptic marker vGat were expressed in cortical layer 1 (Fig. 2, A to C). In acute brain slices from GW 22, pyramidal neurons in deep cortical layers had high membrane resistance (1.1 ± 0.1 gigohms, $n = 15$), low membrane capacitance (23.0 ± 2.9 pF, $n = 15$), and fired only one or two action potentials (Fig. 2, D and E) (19). Cortical neurons expressed GABA_A receptors (fig. S2) and displayed typical GABAergic miniature synaptic currents (Fig. 2F). Thus, human cortical neurons express functional GABA_A receptors and GABAergic synapses in the late second trimester.

We characterized the morphology and location of recorded cortical neurons by filling cells with neurobiotin and postimmunostaining with streptavidin-549 and the deep cortical layer marker Ctip2. Ctip2 marker expression (Fig. 2D and fig. S3) identified layer 5 pyramidal neurons with apical dendrites extending to cortical layer 1 (Fig. 2D). We recorded robust evoked synaptic responses only when stimulating layer 1 (Fig. 2G), but not deep cortical layers (-0.1 ± 0.6 pA,

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Blue light stimulation of layer 1 evoked synaptic responses in layer 4 and layer 5 pyramidal neurons. The light-evoked responses were not sensitive to α -amino-3-hydroxy-5-methyl-4-isoxazole propi-

onate (AMPA) or *N*-methyl-D-aspartate (NMDA) receptor antagonists [6-cyano-2, 3-dihydroxy-7-nitro-quinoxaline (CNQX) and D-2-amino-5-phosphonopentanoate (D-APV), respectively] but were abolished by the GABA_A receptor antagonist bicuculline (BMI) (Fig. 1, J to L) and reversibly blocked by tetrodotoxin (TTX, $n = 4$; Fig. 1K). Stimulation of layer 1 axons also induced GABAergic responses from layer 2/3 neurons, supporting observations that the layer 1 GABAergic plexus connects with pyramidal neurons from multiple layers in neonatal mice (13).

Morphologically, the human brain has abundant GABAergic synapses in cortical layer 1 as early as gestational week (GW) 12 (17, 18). In the second trimester, subplate neurons show spontaneous firing and synaptic activity (19, 20). Examining the expression and distribution of GABAergic axons in human cortex at GW 24, we found that both the axon marker neurofilament-2H3 and the GABAergic presynaptic marker vGat were expressed in cortical layer 1 (Fig. 2, A to C). In acute brain slices from GW 22, pyramidal neurons in deep cortical layers had high membrane resistance (1.1 ± 0.1 gigohms, $n = 15$), low membrane capacitance (23.0 ± 2.9 pF, $n = 15$), and fired only one or two action potentials (Fig. 2, D and E) (19). Cortical neurons expressed GABA_A receptors (fig. S2) and displayed typical GABAergic miniature synaptic currents (Fig. 2F). Thus, human cortical neurons express functional GABA_A receptors and GABAergic synapses in the late second trimester.

We characterized the morphology and location of recorded cortical neurons by filling cells with neurobiotin and postimmunostaining with streptavidin-549 and the deep cortical layer marker Ctip2. Ctip2 marker expression (Fig. 2D and fig. S3) identified layer 5 pyramidal neurons with apical dendrites extending to cortical layer 1 (Fig. 2D). We recorded robust evoked synaptic responses only when stimulating layer 1 (Fig. 2G), but not deep cortical layers (-0.1 ± 0.6 pA,

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$n = 10$) or the subplate (-1.6 ± 0.5 pA, $n = 10$). Evoked responses were insensitive to the AMPA receptor antagonist CNQX but were blocked by BMI (Fig. 2, G and H). The evoked synaptic currents reversed at -34.7 mV, close to the Cl^- equilibrium potential [-31.1 mV = -46.0 mV (by Nernst equation) + 14.9 mV (junction potential)], consistent with mediation through GABA_A receptors (Fig. 2, I and J).

Neurons in human layer 1 cortex had GABAergic responses by GW 20 that were not evident at GW 16 or 18. By GW 22, the majority of deep-layer neurons demonstrated GABAergic synaptic responses. We did not detect evoked responses in GW 24 layer 2/3 neurons, which are still very immature (fig. S4). To evaluate the potential contribution of layer 1 local neurons to the GABAergic response of pyramidal neurons, we applied glutamate locally in layer 1. Although this induced robust firing of layer 1 neurons, it did not evoke synaptic responses in layer 4 and layer 5 pyramidal neurons (fig. S5). Thus, a GABAergic axon plexus arising from long-projection neurons is present in layer 1 in second-trimester human cortex.

GABA responses are depolarizing in the mouse brain during the first postnatal week (fig. S6) as a result of high intracellular chloride ion concentration $[\text{Cl}^-]$ (6, 7). To study GABA responses in

GW 22 cortical neurons, we performed gramicidin-perforated patch recordings to avoid perturbing the intracellular $[\text{Cl}^-]$. GABA responses reversed at -44.4 mV (fig. S7) and were depolarizing because the resting membrane potential of cortical neurons at this stage was -64.8 ± 1.6 mV ($n = 14$). We then monitored intracellular Ca^{2+} in response to GABA by loading neurons with a membrane-permeable Ca^{2+} indicator, Oregon Green BAPTA-1 AM. Local GABA application induced robust intracellular Ca^{2+} elevation in layer 5 cortical neurons (fig. S7). Thus, as in mouse (21–23), GABA is depolarizing in human fetal cortex when functional GABAergic synapses are first established.

To explore the effect of the layer 1 GABAergic axon plexus on cortical development, we selectively blocked synaptic GABA release from mouse ZI neurons by selective expression of tetanus toxin light chain (TeLC), which blocks synaptic vesicle release (24). At P0–P1, we injected AAV virus containing Cre-dependent TeLC fused with 2A-green fluorescent protein under human elongation factor 1a (EF1a) promoter (AAV1-EF1a-DIO-TeLC-2A-GFP) stereotactically into the ZI of $\text{SST}::\text{Cre};\text{Ai14}$ mice. We detected GFP expression in the ZI within 1 week (fig. S8). To test the efficiency of TeLC blockade, we first co-injected AAV1-DIO-ChR2 with AAV1-DIO-TeLC-2A-GFP unilaterally into the

ZI. Blue light stimulation of cortical layer 1 failed to induce synaptic currents in layer 5 pyramidal neurons from acute brain slices obtained one week post viral injection (0.12 ± 0.20 pA, $n = 16$; Fig. 3, A and B). To evaluate the extent to which layer 1 GABAergic synaptic release is impaired by TeLC expression in the ZI, we recorded GABAergic responses in layer 5 pyramidal neurons evoked by electrical stimulation of layer 1. We found that the amplitude of evoked GABAergic responses was reduced by 79.5% in neurons ipsilateral to the TeLC injection site (34.1 ± 1.8 pA, $n = 18$) relative to the contralateral hemisphere (166.0 ± 6.4 pA, $n = 13$, $P < 0.001$; Fig. 3C). Given that TeLC-2A-GFP labeled 70.7% of ZI SST^+ neurons ($n = 1465/2072$; fig. S8), these data suggest that ZI projections provide a major GABAergic synaptic input to layer 1 in the first postnatal week.

We next examined cortical neuron development with ZI GABAergic transmission blocked by unilateral injection of AAV1-DIO-TeLC-2A-GFP into P0–P1 $\text{SST}::\text{Cre}$ mice. The frequency of spontaneous GABAergic and glutamatergic synaptic currents was reduced 1 week after injection (Fig. 3D and fig. S9), but the amplitudes were unchanged (fig. S9). The number of dendritic branches in the apical dendritic tuft was decreased in layer 5 neurons ipsilateral to the TeLC injection. Basal

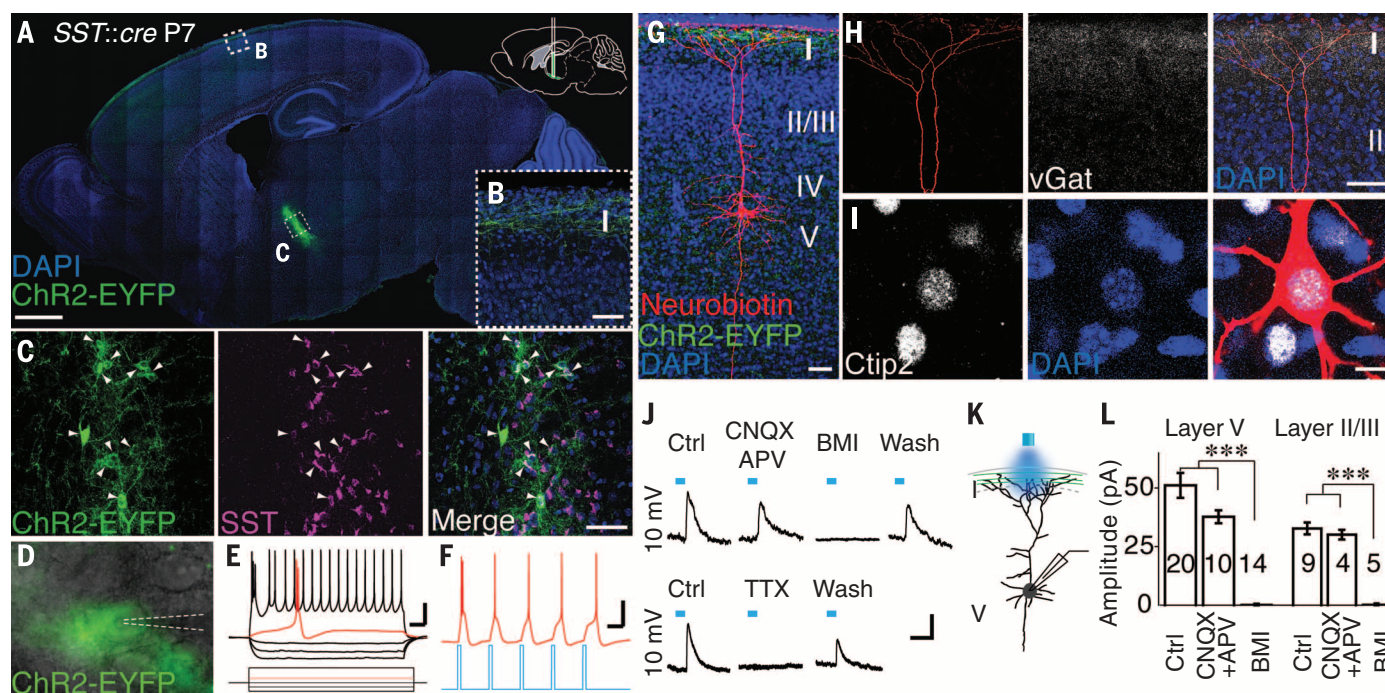


Fig. 1. ZI GABAergic neurons project to cortical layer 1 and form functional synapses with cortical neurons in neonatal mouse brain. (A) Sagittal view of SST^+ ZI neurons specifically labeled with ChR2-EYFP. Locations of images shown in (B) and (C) are indicated. DAPI, 4',6-diamidino-2-phenylindole. (B) Specific labeling of axons in cortical layer 1. (C) ZI EYFP^+ neurons coexpress SST. Arrowheads indicate colocalization. (D) Image of the ZI with superimposed EYFP at P7. (E) Burst-firing of ZI EYFP^+ neurons. (F) Action potentials induced by blue light stimulation. (G) Neurobiotin-labeled layer 5 pyramidal cell in P8 $\text{SST}::\text{cre}$ mouse with ChR2-EYFP injected into the ZI at P0. Note overlap between apical dendritic tufts and EYFP^+ axons in layer 1.

(H) A high density of vGat expression in layer 1 overlaps with the dendritic tuft. (I) Recorded layer 5 neurons coexpress Ctip2. (J) Schematic showing blue light stimulation of layer 1 EYFP^+ axons while recording layer 5 neurons. (K and L) Representative traces (K) and statistical analysis [(L), recorded during P6–P8] show light-evoked synaptic responses at +10 mV with or without receptor antagonists (10 μM CNQX, 50 μM D-APV, 20 μM BMI, or 1 μM TTX). Scale bars, 1 mm (A); 50 μm [(B), (C), (G), and (H)]; 25 mV, 100 ms [(E) and (F)]; 10 μm (I); 20 pA, 50 ms (K). *** $P < 0.001$. Here and in other figures, numerals within each bar of a graph represent sample size; error bars denote SEM.

Fig. 2. Synaptic responses in developing human cortical neurons from layer 1 GABAergic axons. (A to C) Neurofilament-2H3 (NF-2H3) (A) and vGat (B) are co-expressed (C) in human fetal cortical layer 1. (D) Cortical neuron filled intracellularly with neurobiotin. Ctip2, cortical layer 5 marker; DAPI, nuclear marker. (E) Action potential firing of a layer 5 neuron under a stepped-current protocol ranging from -30 pA to 50 pA. (F) Miniature postsynaptic potentials of cortical neurons are sensitive to BMI. (G) Synaptic responses in layer 5 neurons evoked from cortical layer 1 were insensitive to CNQX but were reversibly blocked by BMI. (H) Summarized results showing average amplitude of evoked PSC responses in the presence or absence of BMI. (I and J) Sample traces (I) and summarized result (J) of evoked synaptic responses at holding potentials from -65 mV (bottom) to 35 mV (top), with 20 mV interval. Scale bars, 50 μ m [(A) to (D)]; 25 mV, 200 ms (E); 40 pA, 2 s, 0.2 s (dashed line) (F); 100 pA, 10 ms [(G) and (I)].

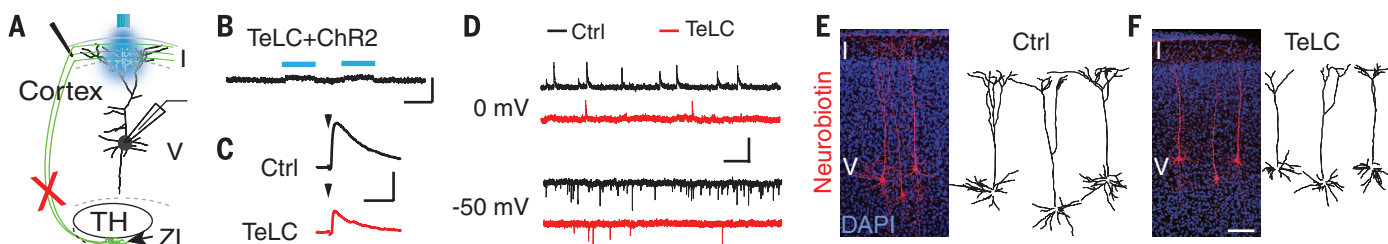
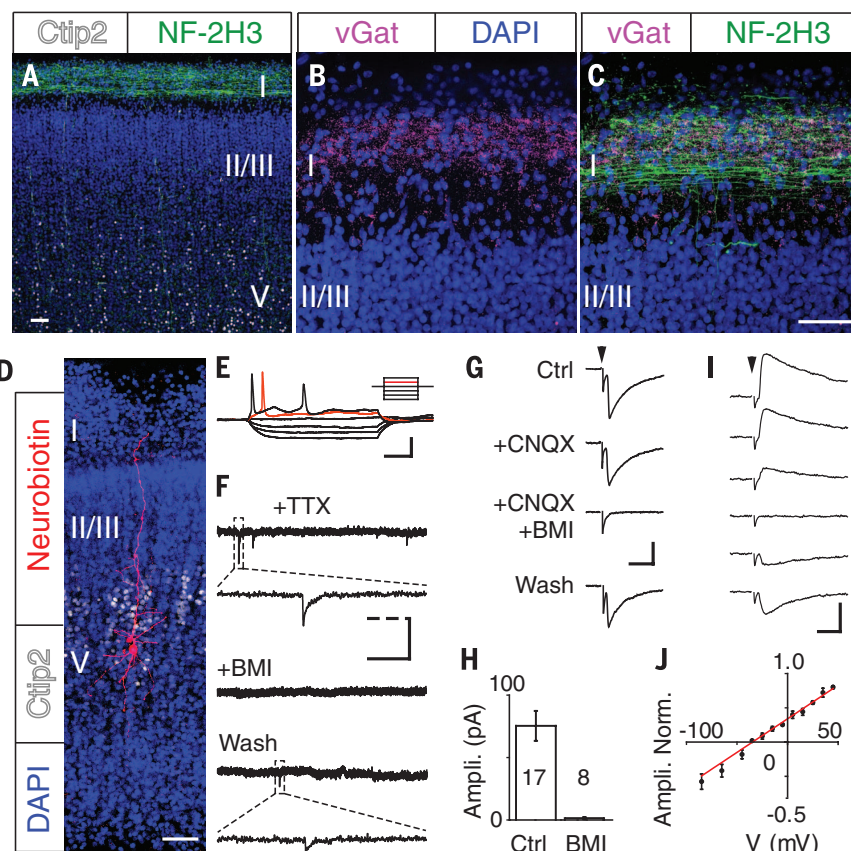


Fig. 3. Reduced synaptic activity and apical dendritic complexity after blocking ZI GABAergic transmission in neonatal mice. (A) Schematic showing layer 5 neurons recorded with light or electrical stimulation of layer 1. Red cross indicates blocked ZI activity. TH, thalamus. (B) Light stimulation of layer 1 fails to induce synaptic responses in P7 SST::Cre mice where TeLC and ChR2 were coexpressed in the ZI (TeLC+ChR2) at P0 (compare to Fig. 1K). (C) Reduced amplitude of layer 1 electrical stimulation-induced GABAergic responses (at 0 mV) in SST::Cre mice expressing TeLC in the ZI. (D) Reduced frequency of spontaneous GABAergic (at 0 mV) and glutamatergic (at -50 mV) responses in layer 5 neurons (P7–P9). (E and F) Morphology of P7 layer 5 cortical neurons in control-injected (E) or TeLC-injected (F) hemisphere. (G and H) Linear Sholl plots of apical (G) or basal (H) dendritic branches, showing best-fit polynomials (dashed lines) and SEM (shaded regions). Statistical results show maximum number of intersections. (I and J) Confocal microscopy images (I) and statistical results (J) show decreased spines in apical dendrites ($***P < 0.001$). Scale bars, 100 pA, 50 ms (B); 20 pA, 50 ms (C); 20 pA, 2 s (D); 100 μ m (F); 5 μ m (I). In (G), (H), and (J), error bars denote SEM.

dendritic branches were unaffected (Fig. 3, E to H). We also observed reduced spine numbers in apical dendrites after TeLC treatment (Fig. 3, I and J). Thus, layer 1 GABAergic synaptic activity is crucial for normal development of synapses and dendrites in pyramidal neurons.

GABA responses become hyperpolarizing after the first postnatal week in mice (fig. S6), due to a lower intracellular $[Cl^-]$, and thus become inhibitory (7, 25–27). To explore whether a functional ZI GABAergic pathway persists in the mature mouse brain, we used selective labeling of ChR2 layer 1 ZI

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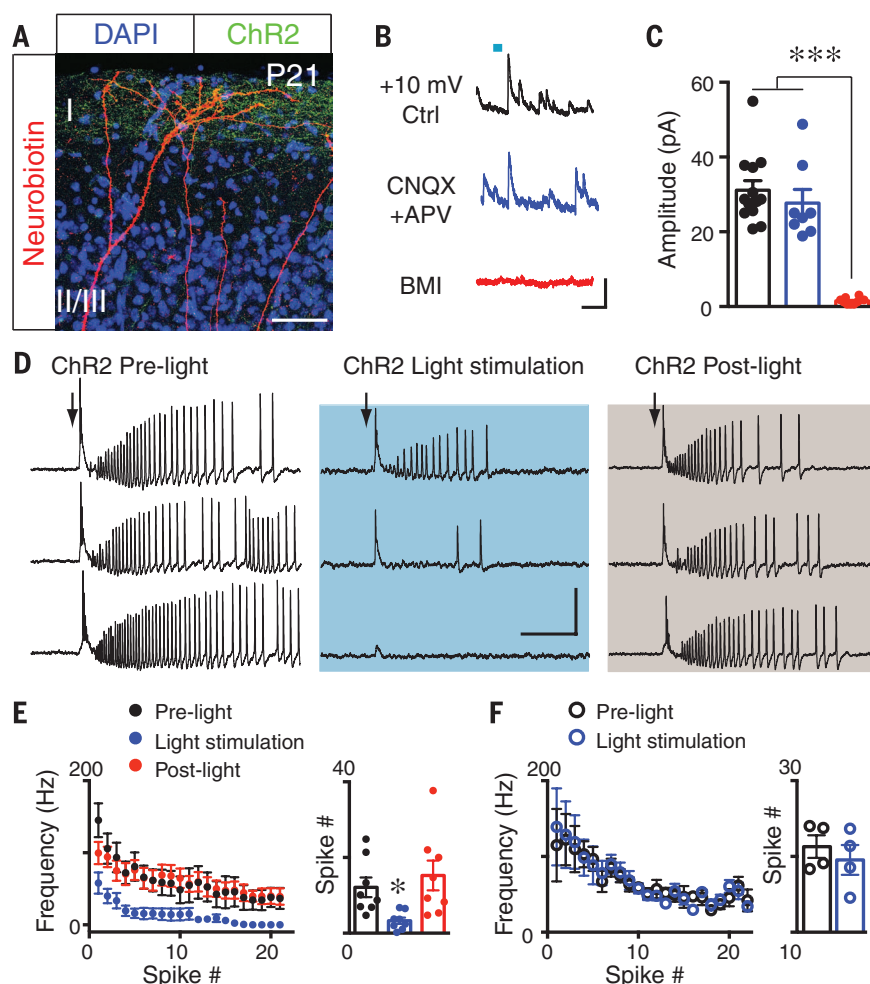


Fig. 4. ZI GABAergic connections remain functional in mature mouse brain and can suppress epileptiform activity. (A) Layer 1 ChR2-EYFP axons overlap with distal dendrites of layer 5 pyramidal neurons in P21 SST::Cre mice. (B and C) Representative (B) and statistical (C) results show that layer 1 light stimulation-induced synaptic currents in layer 5 neurons (at +10 mV) are GABAergic (*** $P < 0.001$). (D) Epileptiform activity is reversibly blocked by light stimulation of layer 1 ChR2-EYFP⁺ axons from ZI (50 ms, 10 Hz, 20 pulses, 30 s interval). (E and F) Both spike frequency and numbers were reversibly reduced upon light stimulation (* $P < 0.05$) (E), but not in controls lacking ChR2 expression (F). Scale bars, 50 μ m (A); 50 pA, 100 ms (B); 40 mV, 0.2 s (D). In (C), (E), and (F), error bars denote SEM.

axons in P21 mice (Fig. 4A) and found that blue light stimulation induced robust GABAergic synaptic currents in pyramidal neurons (Fig. 4, B and C). To evaluate the role of ZI GABAergic projections in cortical circuit function, we used a slice model in which cortical excitation was enhanced by Mg^{2+} -free artificial cerebrospinal fluid (28). We then used cell-attached recordings of cortical layer 4 neurons to preserve intracellular Cl^- integrity. Electrical stimulation of adjacent regions within the same cortical layer induced epileptiform activity in recorded neurons (fig. S10). Coactivation of ZI axons by blue light stimulation of layer 1 ChR2-expressing axons reversibly suppressed the induced epileptiform activity (Fig. 4, D and E) but had no effect in slices from the contralateral hemisphere lacking ChR2 expression (Fig. 4F and fig. S10). Thus, ZI GABAergic projections are inhibitory in the mature mouse brain.

The circuit identified here is one of the earliest to appear in cortical development. While the importance of cortical interneurons to circuit function has been well documented (29, 30), our study shows that the ZI circuit originating in the di-encephalon supports synaptogenesis, apical but not basal dendritic branching (Fig. 3, E to H), and spine development (Fig. 3, I and J) in cortical neurons. In the absence of ZI activity, we found decreased inhibitory postsynaptic current (IPSC)

frequency in upper- and lower-layer neurons; we found decreased excitatory postsynaptic current (EPSC) frequency only in layer 5 neurons (Fig. 3, E to H, and fig. S11), possibly because they receive different presynaptic inputs that could target distinct dendritic domains (31). GABA signaling elevates $[Ca^{2+}]$ (32), which could be restricted to the apical dendritic tuft (33) and may modulate apical dendritic development. Neurotrophic factors such as reelin and semaphorin 3A modulate apical dendritic branching and spine density in an activity-dependent manner (34, 35); thus, it will be interesting to examine whether the developmental effects of ZI activity are mediated by neurotrophic factors (4, 36, 37). Relatively modest structural or functional changes in cortical neurons can have substantial behavioral impacts. The defects in dendritic arborization, spine density, and synaptic activity of cortical neurons observed upon blocking ZI activity resemble those implicated in a variety of neurodevelopmental diseases (9, 38–41); thus, the ZI pathway may play a role in disease etiology (42).

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Supplementary Text
Figs. S1 to S11
References (43–47)

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MICROBIOME

Disease tolerance mediated by microbiome *E. coli* involves inflammasome and IGF-1 signaling

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Infections and inflammation can lead to cachexia and wasting of skeletal muscle and fat tissue by as yet poorly understood mechanisms. We observed that gut colonization of mice by a strain of *Escherichia coli* prevents wasting triggered by infections or physical damage to the intestine. During intestinal infection with the pathogen *Salmonella* Typhimurium or pneumonic infection with *Burkholderia thailandensis*, the presence of this *E. coli* did not alter changes in host metabolism, caloric uptake, or inflammation but instead sustained signaling of the insulin-like growth factor 1/phosphatidylinositol 3-kinase/AKT pathway in skeletal muscle, which is required for prevention of muscle wasting. This effect was dependent on engagement of the NLRC4 inflammasome. Therefore, this commensal promotes tolerance to diverse diseases.

Infections and inflammation lead to profound metabolic alterations that are primarily driven by responses of muscle, fat, and liver tissues (1, 2). Coupled with loss of appetite, dysregulated metabolism can lead to the severe metabolic pathology called wasting syndrome (cachexia). Such wasting constitutes loss of skeletal muscle (with and without adipose tissue depletion), resulting in weight loss (2). Tuberculosis, sepsis, and HIV, as well as inflammatory diseases including colitis (1, 3, 4), can trigger wasting. Wasting can also interfere with therapeutic interventions and cause untreatable morbidity and mortality (5).

Mammals have coevolved with a complex gut microbial community, the gut microbiota, and

depend on the metabolic benefits that it confers on the host (6, 7). Here, we have investigated whether constituents of the microbiota have any protective effect during metabolic dysregulation caused by gut trauma and/or infection.

Intestinal injury and inflammation can cause muscle wasting and inflammatory bowel disease (IBD), and Crohn's disease (CD) patients suffer from muscle wasting (8). Antibiotics cause ecological perturbations in the microbiota (9), and coupling antibiotics with disease models can reveal how specific constituents of the microbiota impact disease. The dextran sulfate sodium (DSS) intestinal injury model is one of the best-studied models of IBD/CD-associated pathologies, including intestinal inflammation, mucus erosion, and microbiota decompartmentalization (10). C57Bl/6 mice treated with DSS exhibited muscle and fat wasting that was associated with anorexia, colonic inflammation, and bloody diarrhea (Fig. 1, A and B, and fig. S1). Administration of the broad-spectrum antibiotic cocktail ampicillin, vancomycin, neomycin, and metronidazole (AVNM) had no significant impact on the severity of DSS-induced wasting in C57Bl/6 mice obtained from Jackson Laboratories (Jax mice)

(fig. S2). Surprisingly, we found that C57Bl/6 from the University of California, Berkeley, colony (CB mice) showed significantly less wasting when DSS was coupled with AVNM treatment (Fig. 1C and fig. S2).

We hypothesized that differences in the microbiota composition between Jax and CB mice may account for the differences we observed in wasting pathogenesis in response to the AVNM cocktail plus DSS. We cultured an AVNM-resistant (AVNM^R) population of bacteria from the ceca of CB mice that was not present in Jax mice (fig. S2). On cohousing with AVNM-treated CB mice, AVNM-treated Jax mice became colonized with AVNM^R bacteria and were now protected from DSS-induced wasting. Hence, protection from wasting and CB-associated AVNM^R bacteria can be horizontally transferred to otherwise wasting-susceptible animals (fig. S2).

We performed culture-independent analysis of amplicons generated by primers specific to the V3 and V4 variable regions of bacterial 16S ribosomal RNA genes of cecal content samples from CB and Jax mice treated with AVNM for 5 days (11). The bacterial communities detected were compared phylogenetically (12). We found that AVNM-treated CB mice hosted large numbers of *Escherichia* spp. compared with AVNM-treated Jax mice (fig. S2). Using culturing techniques, serotyping, and genetic and molecular characterization (12), we isolated a single AVNM^R *Escherichia* from the ceca of CB mice that we classified as an *E. coli* O21:H⁺ strain that was absent from Jax mice (fig. S2) (11). We performed multilocus sequence typing (MLST) by analyzing seven loci of *E. coli* O21:H⁺ (tables S1 and S2) (11) and found this *E. coli* to be a perfect match at these loci for *E. coli* of the strain ST101.

Oral administration of *E. coli* O21:H⁺ resulted in colonization of the intestine of Jax mice but had no significant effect on weight, muscle mass, fat mass, or food consumption under homeostatic conditions (fig. S3). However, on DSS treatment, *E. coli* O21:H⁺ Jax mice showed significantly less wasting than did DSS-treated vehicle mice (Fig. 1D and fig. S4). Jax mice that were administered heat-killed *E. coli* O21:H⁺ or live doses of the commensal strain *E. coli* MG1655 exhibited similar wasting as that of vehicle-control mice (fig. S4). In accordance with our animal protocol, we used >15% weight loss as a clinical end point. We terminated all experiments on the day after challenge

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in which one or more animals exhibited >15% weight loss, then harvested tissues for post-mortem analyses. This time point is consistent for a particular disease model but varies among models owing to different disease kinetics. In all experiments, the number of animals and replication numbers were chosen as statistically necessary and are reported in the figure legends (11).

We monocolonized germ-free Swiss Webster (SW) mice by means of oral gavage with *E. coli* O21:H⁺ or *E. coli* MG1655, treated them with DSS, and monitored wasting. These *E. coli* strains colonized the intestines of SW mice equivalently (fig. S4). Compared with *E. coli* MG1655-colonized Jax mice, *E. coli* MG1655-monocolonized SW mice were more susceptible to DSS, with some animals exhibiting >15% weight loss 5 days after treatment owing to muscle and fat loss (Fig. 1, E and F, and fig. S4). In contrast, *E. coli* O21:H⁺-monocolonized mice did not lose weight (Fig. 1, E and F, and fig. S4). Thus, *E. coli* O21:H⁺-mediated protection does not require other microbiota constituents.

E. coli O21:H⁺ and *E. coli* MG1655 LPS were equivalent in their ability to stimulate the innate immune mediator Toll-like receptor 4 (TLR4)

(fig. S4). *E. coli* O21:H⁺-monocolonized mice were more susceptible to an intraperitoneal challenge of *E. coli* O21:H⁺ compared with *E. coli* MG1655-monocolonized mice that received an intraperitoneal challenge of *E. coli* MG1655 (fig. S4). The canonical proinflammatory cytokines associated with wasting—tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), and IL-6—were not down-regulated in *E. coli* O21:H⁺ mice, nor was there any difference in intestinal inflammation between DSS-treated vehicle and *E. coli* O21:H⁺ mice (fig. S5). Thus, differences in LPS levels, stimulatory capacity, or responsiveness to *E. coli* O21:H⁺ were not responsible for the reduced wasting observed in *E. coli* O21:H⁺ mice on DSS challenge. Likewise, we found no difference in intestinal tissue damage in wasting-susceptible and wasting-protected animals, as indicated by the lack of difference between the mice in epithelial cell loss, hyperplasia, edema, or fibrosis (fig. S5).

Metabolic changes observed during wasting are distinct from those observed during food deprivation, and nutritional interventions cannot reverse wasting (12–14). The DSS-induced anorexic response was equivalent in vehicle-control

and *E. coli* O21:H⁺ animals (fig. S4). The comprehensive laboratory animal monitoring system (CLAMS) revealed no differences in the rates of oxygen consumption or metabolic rate, carbon dioxide production, respiratory exchange ratio, activity, or heat production of DSS-treated animals with and without *E. coli* O21:H⁺ (fig. S6).

In untreated mice, *E. coli* O21:H⁺ had no effect on host survival (carried out to 1 year). On DSS treatment, 70% of Jax mice given *E. coli* O21:H⁺ survived, whereas all of the control Jax mice that were wasting-susceptible died within 12 days of treatment initiation (Fig. 1G).

We previously identified an *E. coli* O21:H⁺ strain in a sepsis model caused by intestinal insult (15); one consequence of sepsis is muscle wasting. Mice in this model and in a model of sepsis induced by intravenous injection with this microbe were protected from wasting (15). We therefore asked whether *E. coli* O21:H⁺ ST101 identified in the current study prevents wasting induced by other microbes.

The oral pathogen *Salmonella* Typhimurium induces wasting of muscle and adipose tissue in Jax mice (Fig. 2, A and B, and fig. S7). *E. coli* O21:

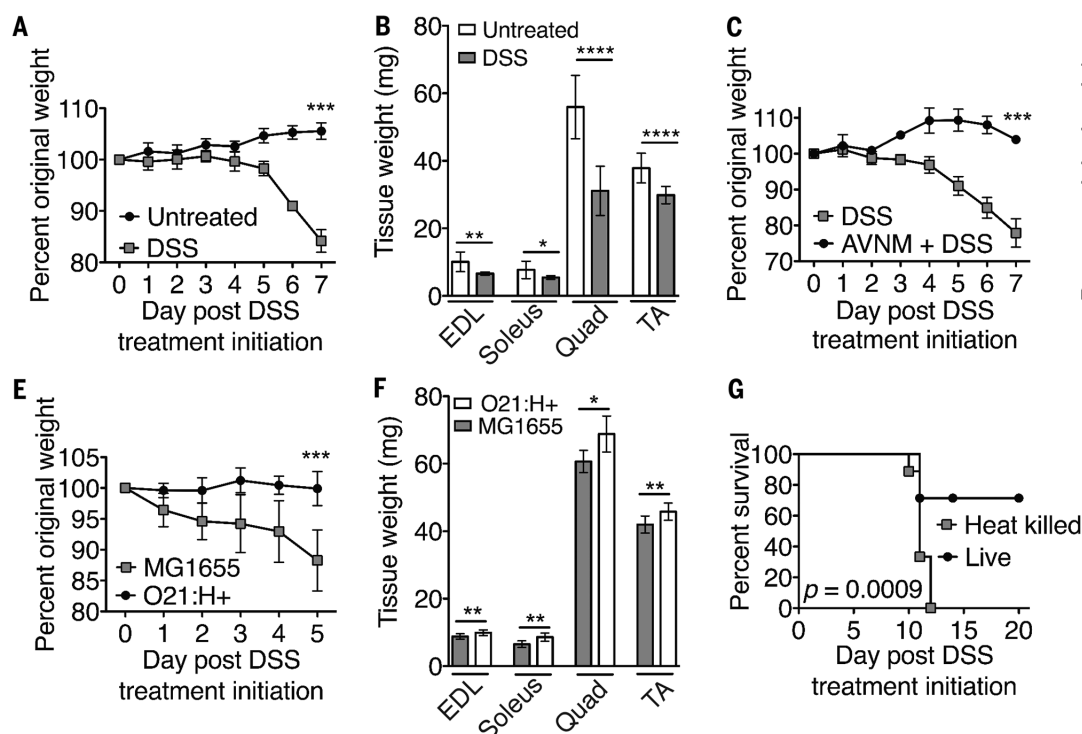


Fig. 1. Effects of *E. coli* O21:H⁺ on DSS-induced skeletal muscle atrophy. (A and B) Jax mice were treated with 5% DSS. (A) Percent original weight. (B) Leg muscle mass at 7 days. Representative experiment ($n = 4$ to 6 independent experiments) using five animals per group, mean $\pm$ S.D. Unpaired t test. (C) Percent original weight of colony-born C57Bl/6 mice (University of California, Berkeley) treated with ampicillin, vancomycin, neomycin, metronidazole, and 5% DSS (methods). Representative experiment ($n = 3$ independent experiments) using four animals per group, mean $\pm$ S.D. Unpaired t test. (D) Percent original weight of 5% DSS-treated Jax mice gavaged with 5×10^8 colony-forming units (CFU) *E. coli* O21:H⁺ or vehicle alone (methods). Representative experiment ($n = 6$ independent experiments) using four animals per group, mean $\pm$ S.D. Unpaired t test. Untreated control mice $\pm$ *E. coli* O21:H⁺ are shown in fig. S3. (E and F) Germ-free Swiss Webster (SW) mice gavaged with 5×10^8 *E. coli* O21:H⁺ or *E. coli* MG1655 (supplementary materials, materials and methods) and treated with 5% DSS. (E) Percent original weight. (F) Leg muscle mass at 5 days from a subset of mice in (E) that were weight matched at day 0. Representative experiment ($n = 4$ independent experiments) using five to seven animals per group, mean $\pm$ S.D. Unpaired t test. Monocolonized SW controls are shown in fig. S3. (G) Percent survival of Jax mice administered *E. coli* O21:H⁺ or heat-killed *E. coli* O21:H⁺ (supplementary materials, materials and methods) and treated with 5% DSS for 7 days, after which DSS was no longer administered. Results are from two independent experiments using four to seven mice per group per experiment. Log rank analysis. Survival analyses of untreated control $\pm$ *E. coli* O21:H⁺ mice have been carried out for 1 year with no deaths from either group. Vehicle/DSS-treated mice exhibit similar death kinetics as heat-killed/DSS treated mice. **** $P < 0.0001$, *** $P < 0.0005$, ** $P < 0.005$, * $P < 0.05$. EDL, extensor digitorum longus; TA, tibialis anterior. Experiments terminated for postmortem analyses at indicated day when one or more mice lost [(A), (B), (D), (E), and (F)] >15% body weight in accordance with our Salk animal protocol or (C) >20% weight loss in accordance with our animal protocol (University of California, Berkeley).

mycin, neomycin, metronidazole, and 5% DSS (methods). Representative experiment ($n = 3$ independent experiments) using four animals per group, mean $\pm$ S.D. Unpaired t test. (D) Percent original weight of 5% DSS-treated Jax mice gavaged with 5×10^8 colony-forming units (CFU) *E. coli* O21:H⁺ or vehicle alone (methods). Representative experiment ($n = 6$ independent experiments) using four animals per group, mean $\pm$ S.D. Unpaired t test. Untreated control mice $\pm$ *E. coli* O21:H⁺ are shown in fig. S3. (E and F) Germ-free Swiss Webster (SW) mice gavaged with 5×10^8 *E. coli* O21:H⁺ or *E. coli* MG1655 (supplementary materials, materials and methods) and treated with 5% DSS. (E) Percent original weight. (F) Leg muscle mass at 5 days from a subset of mice in (E) that were weight matched at day 0. Representative experiment ($n = 4$ independent experiments) using five to seven animals per group, mean $\pm$ S.D. Unpaired t test. Monocolonized SW controls are shown in fig. S3. (G) Percent survival of Jax mice administered *E. coli* O21:H⁺ or heat-killed *E. coli* O21:H⁺ (supplementary materials, materials and methods) and treated with 5% DSS for 7 days, after which DSS was no longer administered. Results are from two independent experiments using four to seven mice per group per experiment. Log rank analysis. Survival analyses of untreated control $\pm$ *E. coli* O21:H⁺ mice have been carried out for 1 year with no deaths from either group. Vehicle/DSS-treated mice exhibit similar death kinetics as heat-killed/DSS treated mice. **** $P < 0.0001$, *** $P < 0.0005$, ** $P < 0.005$, * $P < 0.05$. EDL, extensor digitorum longus; TA, tibialis anterior. Experiments terminated for postmortem analyses at indicated day when one or more mice lost [(A), (B), (D), (E), and (F)] >15% body weight in accordance with our Salk animal protocol or (C) >20% weight loss in accordance with our animal protocol (University of California, Berkeley).

H⁺ Jax mice did not show weight loss, skeletal muscle, or adipose tissue-wasting when infected orally with *S. Typhimurium* compared with vehicle-control infected Jax mice (Fig. 2, C and D, and fig. S7).

We tested whether *E. coli* O21:H⁺ could antagonize wasting in the *Burkholderia thailandensis* pneumonia model in which there is no compromise of the gut barrier. Consistent with critically ill patients with pulmonary dysfunction (16), intranasally infected Jax mice exhibited rapid wasting (~15% weight loss by 2 days after infection) characterized by a depletion of both muscle and fat (Fig. 2, B and E, and fig. S7). Histological analysis showed that the intestinal barrier remained intact during infection in contrast to DSS-treated animals (figs. S5 and S7). To assess gut barrier function, *B. thailandensis*-infected mice were gavaged with fluorescein isothiocyanate (FITC)-dextran, and serum FITC remained negative, confirming that in contrast to FITC-positive serum obtained from DSS-treated mice, the gut

barrier was not breached (fig. S7). *B. thailandensis*-infected/*E. coli* O21:H⁺-colonized Jax mice showed a ~2% decrease in body weight with significantly less muscle and fat wasting than infected mice given vehicle alone, which lost ~15% weight (Fig. 2, D and F, and fig. S7). Consistent with our findings with DSS, *E. coli* O21:H⁺ mitigation of *S. Typhimurium*- and *B. thailandensis*-induced wasting was independent of changes in the infection-induced anorexic response, caloric absorption by the intestine, or alterations in oxygen consumption, carbon dioxide production, activity levels, or heat production (figs. S7 and S8). *E. coli* O21:H⁺ colonization did not result in down-regulation of systemic TNF- α , IL-1 β , or IL-6 or any reduction in tissue damage (*S. Typhimurium*, liver and spleen; *B. thailandensis*, lung, liver, and spleen) (figs. S9 to S11). Consistent with previous reports (17), intestinal pathology in *S. Typhimurium*-infected animals was minimal and indistinguishable in animals with and without *E. coli* O21:H⁺ (fig. S10). The pathogen burdens in *E. coli* O21:H⁺

animals were not reduced compared with infected animals treated with vehicle alone (*B. thailandensis* did not disseminate to the spleen and liver at our doses) (Fig. 2G). Thus, the presence of *E. coli* O21:H⁺ in the intestinal microbiota appears to reduce/inhibit lean body-wasting induced by pathogens or intestinal injury. It is this protective effect, rather than pathogen killing, that significantly promoted survival of *S. Typhimurium*-infected animals given *E. coli* O21:H⁺ (Fig. 2H and fig. S7).

Transcriptional induction of the E3 ubiquitin ligases Atrogin-1 and Murf1 is crucial for muscle atrophy (18–20) and is induced upon challenge with *B. thailandensis*, *S. Typhimurium*, or DSS (Fig. 3A and fig. S12). Induction of *Atrogin-1* and *Murf1* did not occur in the muscle of *E. coli* O21:H⁺ Jax mice challenged with pathogen or DSS and was at equivalent levels found in untreated mice with and without *E. coli* O21:H⁺ (Fig. 3A and fig. S12).

RNA sequencing (RNA-seq) analysis of leg muscles from *S. Typhimurium*-infected mice revealed that components of the insulin/insulin-like

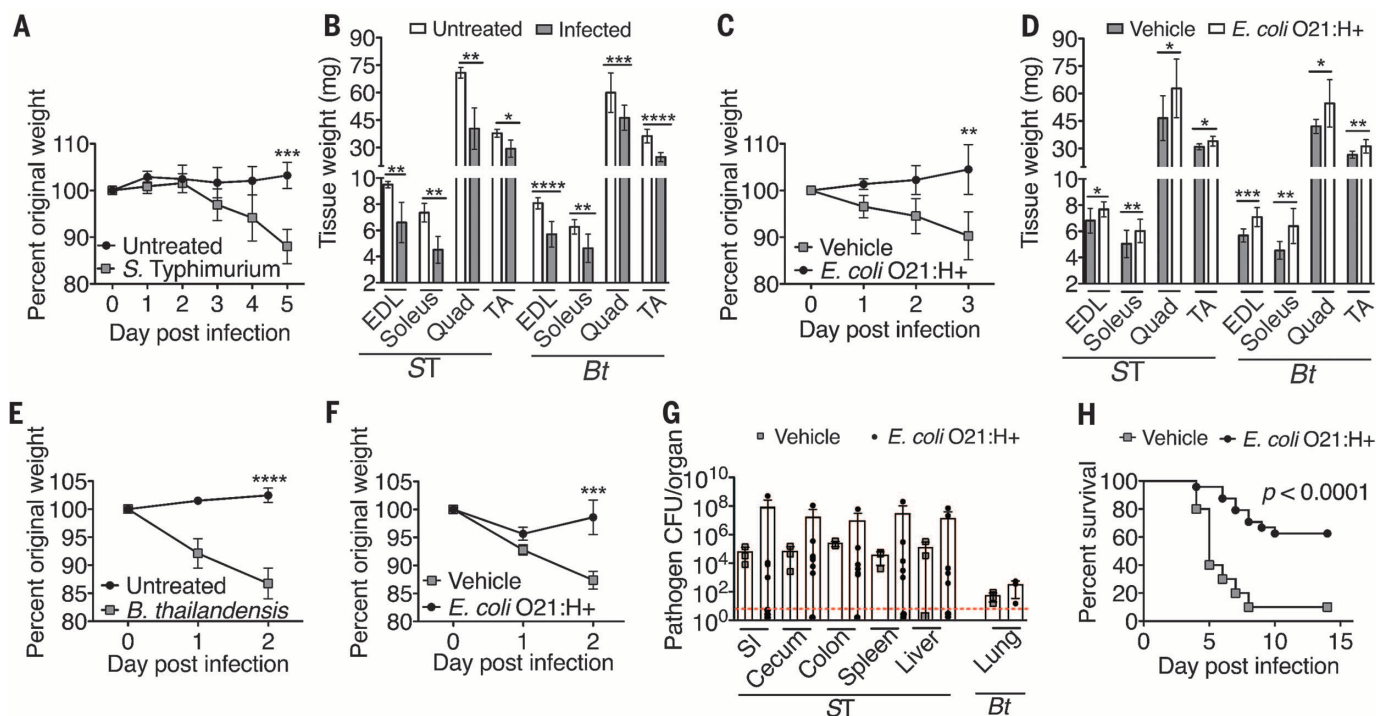


Fig. 2. Effects of *E. coli* O21:H⁺ colonization on muscle wasting induced by infections. (A) Percent original weight of Jax mice orally infected with 8.5 × 10⁶ *S. Typhimurium*. Representative experiment ($n = 3$ independent experiments) using five animals per group, mean ± SD. Unpaired t test. (B) Leg muscle mass from *S. Typhimurium*-infected mice in (A) at day 5 (ST) or *B. thailandensis*-infected mice in (E) at day 2 (Bt). Representative experiment ($n = 3$ independent experiments) using five animals per group, shown as mean ± SD. Unpaired t test. (C) Percent original weight of Jax mice gavaged with 5 × 10⁸ CFU *E. coli* O21:H⁺ or vehicle alone and orally infected with 2 × 10⁷ *S. Typhimurium*. Representative experiment ($n = 7$ independent experiments) using four to five animals per group, mean ± S.D. Unpaired t test. Untreated control mice ± *E. coli* O21:H⁺ are shown in fig. S3. (D) Leg muscle mass from infected *E. coli* O21:H⁺ or vehicle-control mice from (C) at day 3 (ST) and from (F) at day 2 (Bt). Representative experiment ($n = 3$ to 7 independent experiments) using four to five animals per group, mean ± SD. Unpaired t test. Control mice ± *E. coli* O21:H⁺ are shown in fig. S3. (E and F) Jax mice

were (E) left untreated or (F) gavaged with 5 × 10⁸ CFU *E. coli* O21:H⁺ or vehicle alone (supplementary materials, materials and methods) and intranasally infected with 2500 CFU *B. thailandensis*. Representative experiment ($n = 3$ to 7 independent experiments) using four to five animals per group, shown as mean ± SD. Unpaired t test. (G) *S. Typhimurium* (2 × 10⁷ CFU inoculum) or *B. thailandensis* (2500 CFU inoculum) burdens in target tissues from infected mice ± *E. coli* O21:H⁺ at 3 days (ST) or 2 days (Bt). *B. thailandensis* was not detected in the liver and spleen at this dose. Representative experiment ($n = 3$ to 7 independent experiments) using three to seven animals per group, mean ± SD. Unpaired t test. Dotted line indicates limit of detection. (H) Percent survival of Jax mice treated with 5 × 10⁸ CFU *E. coli* O21:H⁺ or vehicle-control orally infected with 2 × 10⁷ *S. Typhimurium*. Results are from two independent experiments with 10 to 12 animals per group per experiment. Log rank analysis. **** $P < 0.0001$ *** $P < 0.0005$, ** $P < 0.005$, * $P < 0.05$. Postmortem analyses at day when one or more mice lost >15% body weight. EDL, extensor digitorum longus; TA, tibialis anterior.

growth factor-1 (IGF-1) signaling pathway, muscle physiology, and metabolism were up-regulated in *E. coli* O21:H⁺ mice compared with infected vehicle-control mice (fig. S13). The IGF-1/phosphatidylinositol 3-kinase (PI3K)/AKT pathway is a master regulator of muscle size that activates factors for protein synthesis and regeneration as well as the down-regulation of *Atrogin-1* and *Murf1* expression in atrophying muscle (21–23). Untreated Jax mice with or without *E. coli* O21:H⁺ had comparable levels of serum IGF-1 as measured by means of enzyme-linked immunosorbent assay (fig. S13) (11) and showed no muscle hypertrophy (fig. S3); likewise when infected with *S. Typhimurium* or *B. thailandensis* or given DSS, serum levels of IGF-1 and downstream signaling components in muscle [IGF-1 receptor (IGFR) and AKT] in *E. coli* O21:H⁺ mice showed sustained activation levels comparable with those of untreated animals, whereas activation levels in challenged animals given vehicle alone

decreased significantly (Fig. 3B and figs. S13 and S14).

IGF-1 is produced primarily by the liver in response to growth hormone (GH) (24). We found that serum levels of GH, liver *Igf-1* expression, and IGF-1 protein levels in the liver, muscle, and intestine were unchanged in challenged *E. coli* O21:H⁺ animals compared with challenged animals treated with vehicle alone or unchallenged animals with and without *E. coli* O21:H⁺ (fig. S13). Instead, during challenge with pathogens or DSS, white adipose tissue (WAT) from challenged *E. coli* O21:H⁺ mice showed higher levels of IGF-1 as compared with WAT from challenged vehicle-control animals and unchallenged animals with and without *E. coli* O21:H⁺ (Fig. 3C and fig. S15). This was associated with *E. coli* O21:H⁺ colonization of the WAT, but not the muscle or WAT-associated lymph nodes (Fig. 3D and fig. S15); neither *B. thailandensis* nor *S. Typhimurium* infected the WAT. Thus, sustained systemic

levels of IGF-1 by *E. coli* O21:H⁺ are associated with increased WAT IGF-1 levels and colonization of the WAT by this commensal. A role for WAT-derived IGF—eliciting systemic effects—is supported by previous studies (25).

Systemic administration of an IGF-1–neutralizing antibody to *E. coli* O21:H⁺ mice in the absence of pathogen infection had no effect on mouse weight (fig. S15). When given the neutralizing antibody intraperitoneally, *E. coli* O21:H⁺ mice infected with *B. thailandensis* were no longer protected from wasting (Fig. 3, E and F, and fig. S15). These animals showed significant weight loss, muscle depletion (but not fat loss), a decrease in muscle IGF-1 signaling, and an increase in muscle expression of *Atrogin-1* and *Murf1* compared with infected *E. coli* O21:H⁺ Jax mice given an immunoglobulin G (IgG) isotype control antibody (Fig. 3, E to H, and figs. S15 and S16). There was no significant difference in the ceca levels of *E. coli* O21:H⁺ or of *B. thailandensis* lung infection levels

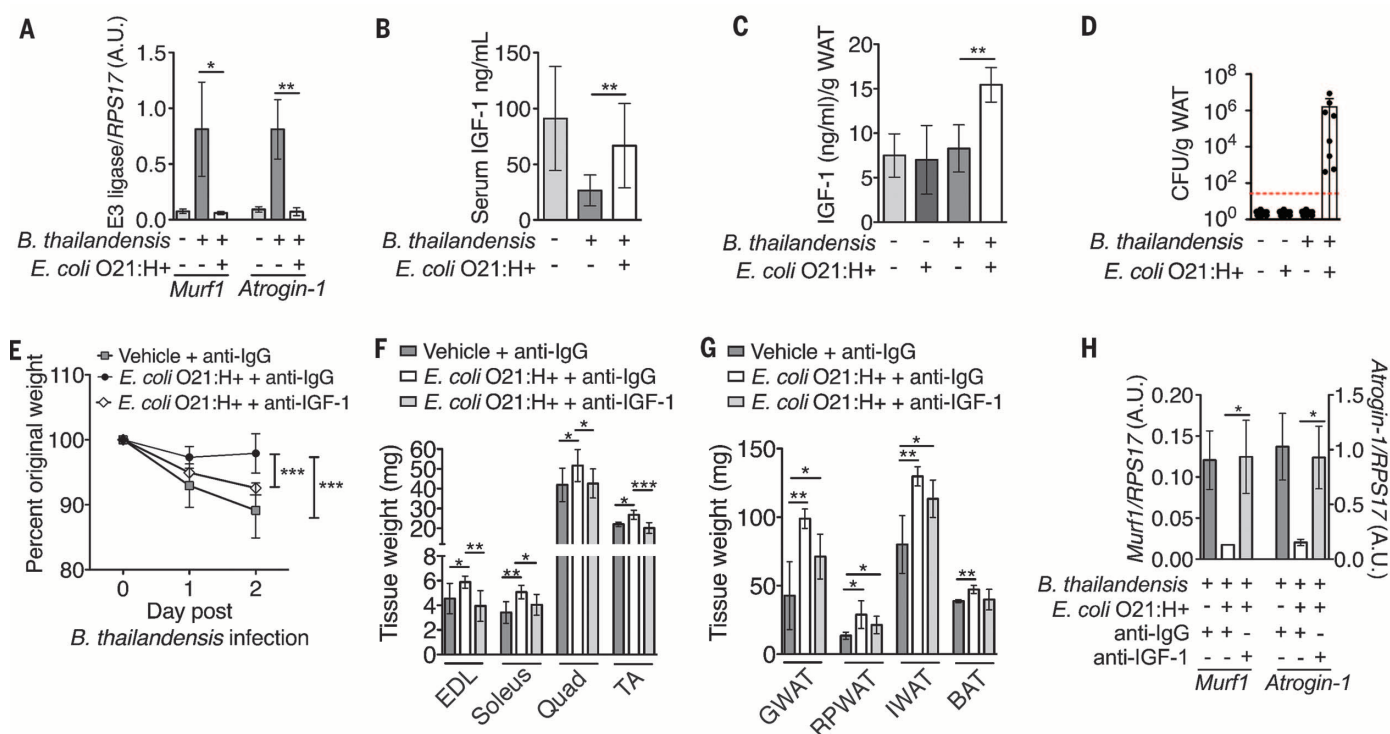


Fig. 3. Gut colonization by *E. coli* O21:H⁺ associated with down-regulation of muscle atrophy programs and maintenance of IGF-1 signaling during infection. (A and B) Jax mice gavaged with 5×10^8 CFU *E. coli* O21:H⁺ or vehicle alone and intranasally infected with 1000 CFU *B. thailandensis*. (A) *Murf1* and *Atrogin-1* expression in leg muscle at 2 days after infection normalized to ribosomal protein S17 (RPS17) expression. Representative experiment ($n = 3$ independent experiments) using four to nine animals per group, mean $\pm$ SD [analysis of variance (ANOVA)]. (B) Serum IGF-1 at 2 days. Representative experiment ($n = 3$ independent experiments) using seven to nine animals per group, shown as mean $\pm$ SD (ANOVA). Additional controls are provided in fig. S14. (C and D) Jax mice were given 5×10^8 CFU *E. coli* O21:H⁺ or vehicle alone and intranasally infected with 2500 CFU *B. thailandensis*. (C) IGF-1 levels in WAT and (D) *E. coli* O21:H⁺ levels in the WAT at day 2. Representative experiment ($n = 4$ independent experiments) using six to ten animals per group, mean $\pm$ SD (ANOVA). Red line indicates the limit of detection. (E to H) Mice given 5×10^8 CFU *E. coli* O21:H⁺

or vehicle alone were injected intraperitoneally with antibody to IGF-1 or IgG isotype control antibody and intranasally infected with 1000 CFU *B. thailandensis*. (E) Percent original weight. Results are from two independent experiments using six to nine mice per group per experiment (12 to 17 mice total), mean $\pm$ SD (ANOVA). Additional controls are provided in fig. S14. (F) Leg muscle mass and (G) adipose tissue mass at 2 days. EDL, extensor digitorum longus; TA, tibialis anterior; GWAT, gonadal white adipose tissue; RPWAT, retroperitoneal WAT; IWAT, inguinal WAT; BAT, brown adipose tissue. Results are from the two independent experiments described in (E) using five to nine mice weight-matched at day 0 (~ 18 g) for each group for each experiment (10 to 14 mice total), mean $\pm$ SD (ANOVA). (H) *Murf1* and *Atrogin-1* expression in leg muscle at 2 days and normalized to RPS17. Representative experiment ($n = 2$ independent experiments) using five to nine animals per group, mean $\pm$ SD (ANOVA). **** $P < 0.0001$, *** $P = 0.0006$, ** $P < 0.005$, * $P < 0.05$. Post-mortem analyses were performed at day when one or more mice lost $>15\%$ body weight.

in *E. coli* O21:H⁺ mice that were treated with antibody to IGF-1 or the IgG isotype control (fig. S15).

The inflammasome—a cytoplasmic multiprotein complex required for activation of the caspase-1 (CASP1) protease (26)—has recently emerged as a critical regulator of host-microbiota interactions and metabolism (26, 27). We therefore examined *Casp1*^{-/-} mice, which are also deficient for CASP11 (28). In contrast to wild-type mice, oral administration of *E. coli* O21:H⁺ to *B. thailandensis*-infected *Casp1*^{-/-} mice did not protect against muscle wasting, the up-regulation of *Murfi* and *Atrogin-1* in muscle, or an infection-induced drop in systemic IGF-1 (Fig. 4, A to C, and fig. S17). Similarly, IGF-1 levels in the WAT were less in *B. thailandensis*-infected *Casp1*^{-/-} mice than in infected wild-type *E. coli* O21:H⁺ mice (fig. S17). Lung infection levels of *B. thailandensis* and intestinal levels of *E. coli* O21:H⁺ were not signif-

icantly different between infected *Casp1*^{-/-} and wild-type mice with or without *E. coli* O21:H⁺ (fig. S17).

Several distinct inflammasomes have been described, each of which contains a protein of the nucleotide-binding domain-containing (NLR) protein superfamily. The NLRC4 inflammasome is activated by the inner rod protein of type three secretion systems (TTSSs) and by flagellin of Gram-negative bacteria (26), both of which are encoded by *E. coli* O21:H⁺ (table S2). Similarly to *Casp1*^{-/-} mice, the presence of *E. coli* O21:H⁺ in *B. thailandensis*-infected *Nlr4*^{-/-} mice did not antagonize wasting or prevent an infection-induced drop in systemic IGF-1 levels (Fig. 4D and fig. S18). Colonization levels of *E. coli* O21:H⁺ in the WAT deposits of *B. thailandensis*-infected *Casp1*^{-/-} were equivalent to those in the WAT of infected wild-type mice (fig. S17). There-

fore, the inflammasome is not necessary for translocation of *E. coli* O21:H⁺ from the intestine to the WAT.

Activation of any inflammasome leads to maturation of the proinflammatory cytokines IL-1 β and IL-18. Systemic levels of IL-18 in *B. thailandensis*-infected *E. coli* O21:H⁺ wild-type mice were increased compared with infected vehicle-control mice, with no differences in IL-1 β levels (Fig. 4E and fig. S5). The protective effect of *E. coli* O21:H⁺ against *B. thailandensis*-induced muscle- and fat-wasting occurred in *Il1 β* ^{-/-} mice but not in *Il1 β* ^{-/-} *Il18*^{-/-} mice (fig. S18). *B. thailandensis*-infection levels in the lung and *E. coli* O21:H⁺ levels in the intestine and WAT were similar between infected wild-type and *Il1 β* ^{-/-} *Il18*^{-/-} mice given *E. coli* O21:H⁺ (fig. S18). When *Il1 β* ^{-/-} *Il18*^{-/-} mice were challenged with *B. thailandensis*, IGF-1 loss was not prevented by *E. coli* O21:H⁺ (Fig. 4F). We conclude that the ability of *E. coli* O21:H⁺ to maintain levels of IGF-1 during challenge is mediated by the NLRC4 inflammasome via IL-18 (with a possible synergistic role for IL-1 β) and thereby prevents muscle-wasting and promotes disease tolerance.

The intestinal microbiota performs essential functions for its host to achieve metabolic homeostasis. Our results strongly suggest that a specific constituent of the mouse intestinal microbiota, *E. coli* O21:H⁺, antagonizes wasting triggered by infections and intestinal injury. During homeostasis, *E. coli* O21:H⁺ remains in the intestine, has no effect on weight, body composition, or IGF-1 levels. During challenge, *E. coli* O21:H⁺ sustains systemic IGF-1 and signaling of IGF-1 in muscle to prevent wasting and promote disease tolerance (29–31) independent of metabolic changes, food consumption, caloric uptake, inflammation, or tissue damage. This protection is associated with challenge-induced translocation of *E. coli* O21:H⁺ to WAT and activation of the NLRC4 inflammasome that correlates with increased production of IGF-1 by WAT. The ability to translocate to and colonize the WAT distinguishes *E. coli* O21:H⁺ from other microbes (including *B. thailandensis* and *S. Typhimurium*) that can activate inflammasomes where its presence antagonizes wasting in an inflammasome-dependent manner, possibly induced by its flagellin or the inner rod protein of its TTSS, eprJ. (fig. S19).

The microbiota is crucial for the development and maintenance of a robust immune system and resistance defenses (32, 33). Discovery of a molecular mechanism by which the microbiota can promote tolerance to infection provides a perspective into the evolutionary forces that have driven the coevolution of host-microbiota interactions.

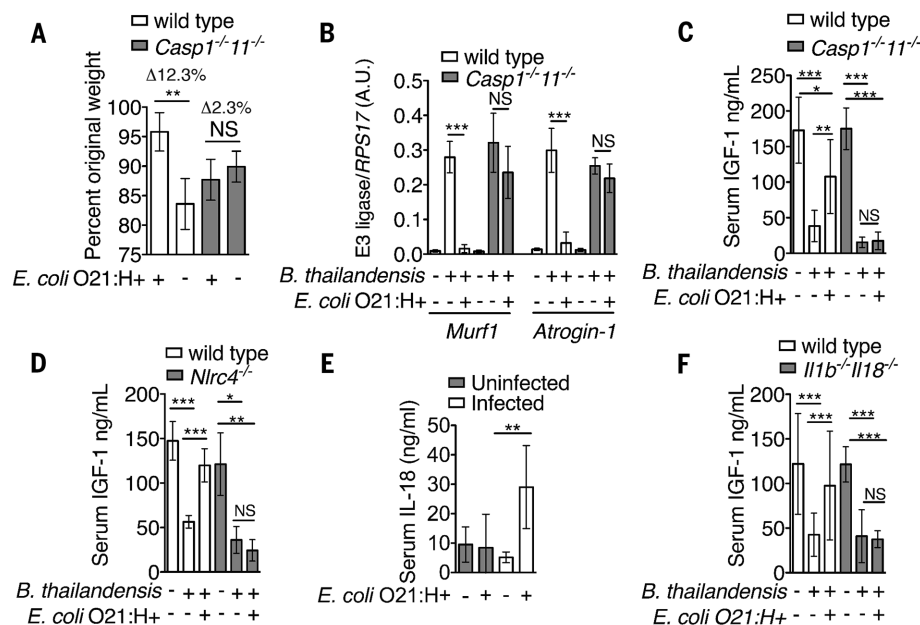


Fig. 4. The Nlr4 inflammasome is required for sustaining IGF-1 levels and mediates the *E. coli* O21:H⁺-associated protection against muscle wasting promoted by pathogenic infection. (A to C) Wild-type and *Casp1*^{-/-} mice were administered 5×10^8 CFU *E. coli* O21:H⁺ or vehicle alone and then intranasally infected with 2500 CFU *B. thailandensis* as described in the supplementary materials, materials and methods. (A) Percent original weight at day 2. Additional controls are provided in fig. S14. (B) Expression of *Murfi* and *Atrogin-1* was determined in leg muscle at 2 days after infection normalized to expression levels of RPS17. (C) Serum IGF-1 quantified at day 2. Representative experiment ($n = 3$ independent experiments) using 10 animals per wild-type group and five to seven mice per *Casp1*^{-/-} group, mean $\pm$ S.D. (ANOVA). (D) Wild-type and *Nlr4*^{-/-} mice were given 5×10^8 CFU *E. coli* O21:H⁺ or vehicle alone and infected intranasally with 2500 CFU *B. thailandensis*. Serum levels of IGF-1 at 2 days after infection were measured. Representative experiment ($n = 2$ independent experiments) using 10 animals per wild-type group and five mice per *Nlr4*^{-/-} group, shown as mean $\pm$ SD (ANOVA). (E) Jax mice were given 5×10^8 CFU *E. coli* O21:H⁺ or vehicle alone and then intranasally infected with 2500 CFU *B. thailandensis* as described in the supplementary materials, materials and methods, and serum IL-18 levels were measured at 2 days after infection. Representative experiment ($n = 2$ independent experiments) using 10 animals per group, mean $\pm$ SD (ANOVA). (F) Wild-type and *Il1 β* ^{-/-} *Il18*^{-/-} mice were administered 5×10^8 CFU *E. coli* O21:H⁺ or vehicle alone and then intranasally infected with 2500 CFU *B. thailandensis*. Serum levels of IGF-1 at 2 days after infection were measured. Representative experiment ($n = 3$ independent experiments) using 10 animals per wild-type group and eight mice per *Il1 β* ^{-/-} *Il18*^{-/-} group, mean $\pm$ SD (ANOVA). *** $P < 0.0003$, ** $P < 0.006$, * $P < 0.05$. Experiment was terminated at 2 days for postmortem analyses when one or more mice lost >15% body weight in accordance with our Salk animal protocol. Additional controls for (C), (D), and (F) are provided in fig. S14.

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SUPPLEMENTARY MATERIALS

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VIROLOGY

Retroviruses use CD169-mediated trans-infection of permissive lymphocytes to establish infection

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Dendritic cells can capture and transfer retroviruses in vitro across synaptic cell-cell contacts to uninfected cells, a process called trans-infection. Whether trans-infection contributes to retroviral spread in vivo remains unknown. Here, we visualize how retroviruses disseminate in secondary lymphoid tissues of living mice. We demonstrate that murine leukemia virus (MLV) and human immunodeficiency virus (HIV) are first captured by sinus-lining macrophages. CD169/Siglec-1, an I-type lectin that recognizes gangliosides, captures the virus. MLV-laden macrophages then form long-lived synaptic contacts to trans-infect B-1 cells. Infected B-1 cells subsequently migrate into the lymph node to spread the infection through virological synapses. Robust infection in lymph nodes and spleen requires CD169, suggesting that a combination of fluid-based movement followed by CD169-dependent trans-infection can contribute to viral spread.

To understand how retroviruses disseminate in secondary lymphoid organs of living animals, we introduced fluorescently labeled murine leukemia virus (MLV) or human immunodeficiency (HIV) subcutaneously (s.c.) into footpads of anesthetized mice and monitored their arrival at the draining popliteal lymph node (pLN). MLV and HIV viruses accumulated at the floor of the subcapsular sinus (SCS), where the lymph node cortex faces the arriving lymphatic fluid (Fig. 1, A and B, and movies S1 and S2). MLV Gag-GFP (green fluorescent protein) viruses accumulated particularly at the SCS above B cell follicles and persisted for >6 hours, whereas beads conjugated with antibodies to the complement receptor 1/2 entered B cell follicles, as previously described (1–3) (Fig. 1A and fig. S1). The cell type responsible for MLV capture (GFP⁺) consisted mostly of CD169⁺ CD11b⁺ macrophages (~80%) (Fig. 1C and fig. S2) (4, 5). Within the pLN tissue, MLV Gag-GFP and HIV Gag-GFP were solely associated with CD169⁺ cells (Fig. 1D, figs. S3 and S4, and movie S3). No overlap between CD169 and CD3, CD19, or CD11c was

observed (fig. S5). When pLN macrophages were depleted, MLV capture was severely compromised (fig. S6).

These data indicate that MLV and HIV are captured in vivo at the pLN predominantly by CD169⁺ CD11b⁺ macrophages. The ability to capture and transmit HIV to permissive CD4⁺ T cells has previously been associated with dendritic cells (DCs), and two distinct mechanisms have been proposed (6, 7). First, by expressing C-type lectins such as DC-SIGN, DCs can capture HIV by recognizing the viral envelope glycoprotein (Env) (7, 8). Second, upon activation, DCs express the immunoglobulin (I)-type lectin CD169/Siglec-1 that can bind MLV and HIV through the recognition of sialyllactose on gangliosides that are embedded in the viral lipid membrane (9–12). To distinguish between both mechanisms, we first injected equal amounts of wild-type or mutant MLV lacking the viral envelope glycoprotein (ΔEnv) into the footpad of C57BL/6 mice, but observed no difference in virus capture at the draining pLN (Fig. 1E). In contrast, depletion of gangliosides in the retrovirus membrane (13) reduced MLV capture, suggesting a role for CD169/Siglec-1 (Fig. 1F). Indeed, a single injection of antibodies to CD169 before the administration of MLV and HIV impaired virus capture at the pLN SCS floor in C57BL/6 mice (Fig. 1, G and H, and fig. S7, A and B). MLV and HIV capture at pLNs were also significantly reduced in CD169 knockout mice (*Siglec1*^{−/−}) (14) compared to C57BL/6 mice (Fig. 1, I and J, and fig. S7C) (15). Blockade of the mouse DC-SIGN homolog SIGN-RI (16) had no effect on MLV capture in vivo (fig. S8).

CD169-expressing macrophages are frequently located at the borders between circulating fluids such as the lymph and blood, and lymphoid structures similar to those seen in LNs are also

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observed in the marginal zone of the spleen (Fig. 1K) (17). Indeed, blood-derived MLV Gag-GFP particles were captured at the marginal zone by CD169⁺ macrophages (Fig. 1K and fig. S9). MLV capture at the spleen was significantly reduced by CD169-targeting antibodies, as well as

in *Siglec1*^{-/-} mice (Fig. 1, L and M). To similarly study early events during HIV infection, we used the BLT humanized mouse model, which exhibits good reconstitution of human macrophages and T cells (18). Fluorescently labeled HIV injected intravenously (i.v.) into humanized mice was as-

sociated with CD169⁺ CD11b⁺ macrophages in the engrafted CD45⁺ human cell population of the spleen (fig. S10). Blocking CD169 significantly reduced association between HIV and splenocytes (Fig. 1N and fig. S11). These data document that mouse and human CD169⁺ macrophages efficiently

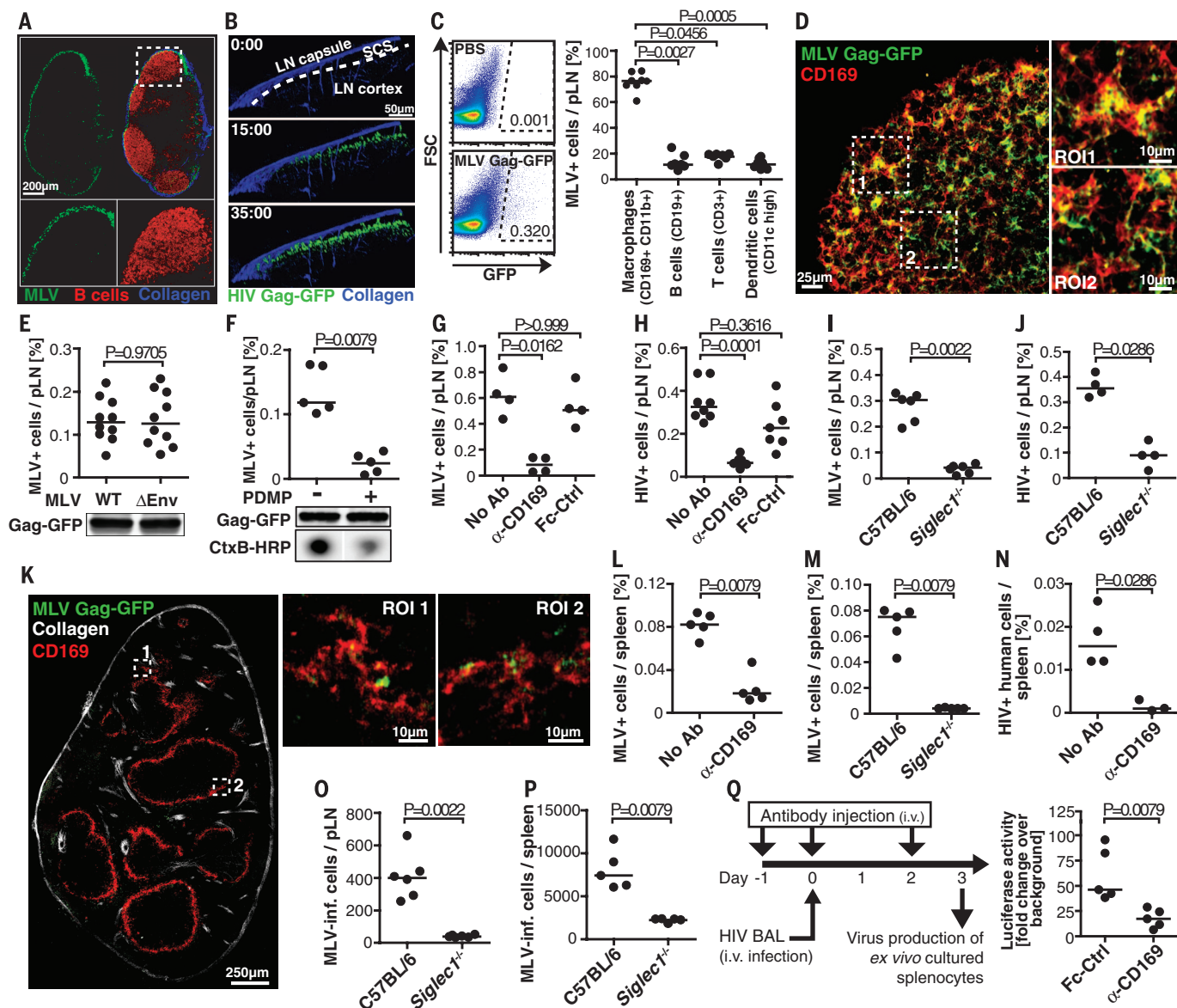


Fig. 1. MLV and HIV infection are facilitated by CD169/Siglec1-expressing macrophages. (A) Immunohistochemistry of a pLN section 0.5 hours after s.c. injection of MLV Gag-GFP (green) into a C57BL/6 mouse. Red, B cells; blue, collagen. (B) Image sequence (movie S2) of HIV Gag-GFP (green) capture at pLN SCS floor (collagen, blue) after s.c. injection into C57BL/6 mouse. Time in minutes. (C) Quantification of MLV Gag-GFP capture at pLNs (n = 7 to 9) 1 hour after s.c. injection. MLV-binding (GFP⁺) cell types were identified by the indicated markers. FSC: front scatter; PBS: phosphate-buffered saline. (D) Image of MLV (green) capture by CD169-expressing macrophages (red) at the pLN SCS floor. CD169⁺ macrophages were stained by s.c. injection of CD169-CF568 antibodies. (E and F) Quantification of MLV capture at pLNs 1 hour after s.c. injection into C57BL/6 mice for wild-type (WT, n = 10), mutant (ΔEnv, n = 10), or ganglioside-depleted MLV Gag-GFP [PDMP⁺ (1-phenyl-2-decanoylamino-3-morpholino-1-propanol), n = 5]. Total amounts of Gag-GFP were estimated by Western blotting and ganglioside depletion by cholera toxin

B-horseradish peroxidase (CtB-HRP) staining. (G and H) MLV Gag-GFP (n = 4) and HIV Gag-GFP (n = 7 to 8) capture at the pLNs of C57BL/6 mice after injection of CD169 or control (Ctrl) antibodies. (I and J) MLV Gag-GFP (n = 6) and HIV Gag-GFP (n = 4) capture at the pLNs of C57BL/6 and *Siglec1*^{-/-} mice. (K) Immunohistochemistry of a spleen section 0.5 hours after i.v. injection of MLV Gag-GFP (green). Red, CD169; gray, collagen. (L and M) MLV Gag-GFP capture at the spleen after i.v. injection of CD169 antibodies (n = 5) or in *Siglec1*^{-/-} mice (n = 5). (N) HIV Gag-GFP capture at the spleen of humanized BLT mice in the absence or presence of CD169 antibodies (n = 3 to 4). (O and P) MLV-infected cells in pLNs (n = 6) and spleen (n = 5) of C57BL/6 and *Siglec1*^{-/-} mice 2 days after infection. (Q) HIV-infected splenocytes in humanized HSC mice injected with CD169 antibodies (n = 5). HIV production of ex vivo-cultured splenocytes was quantified with TZM-bl reporter cells. Median is shown in all cases. Kruskal-Wallis test followed by Dunn's posttest [(C), (G), and (H)]; Mann-Whitney test for (E), (F), and (I) to (Q).

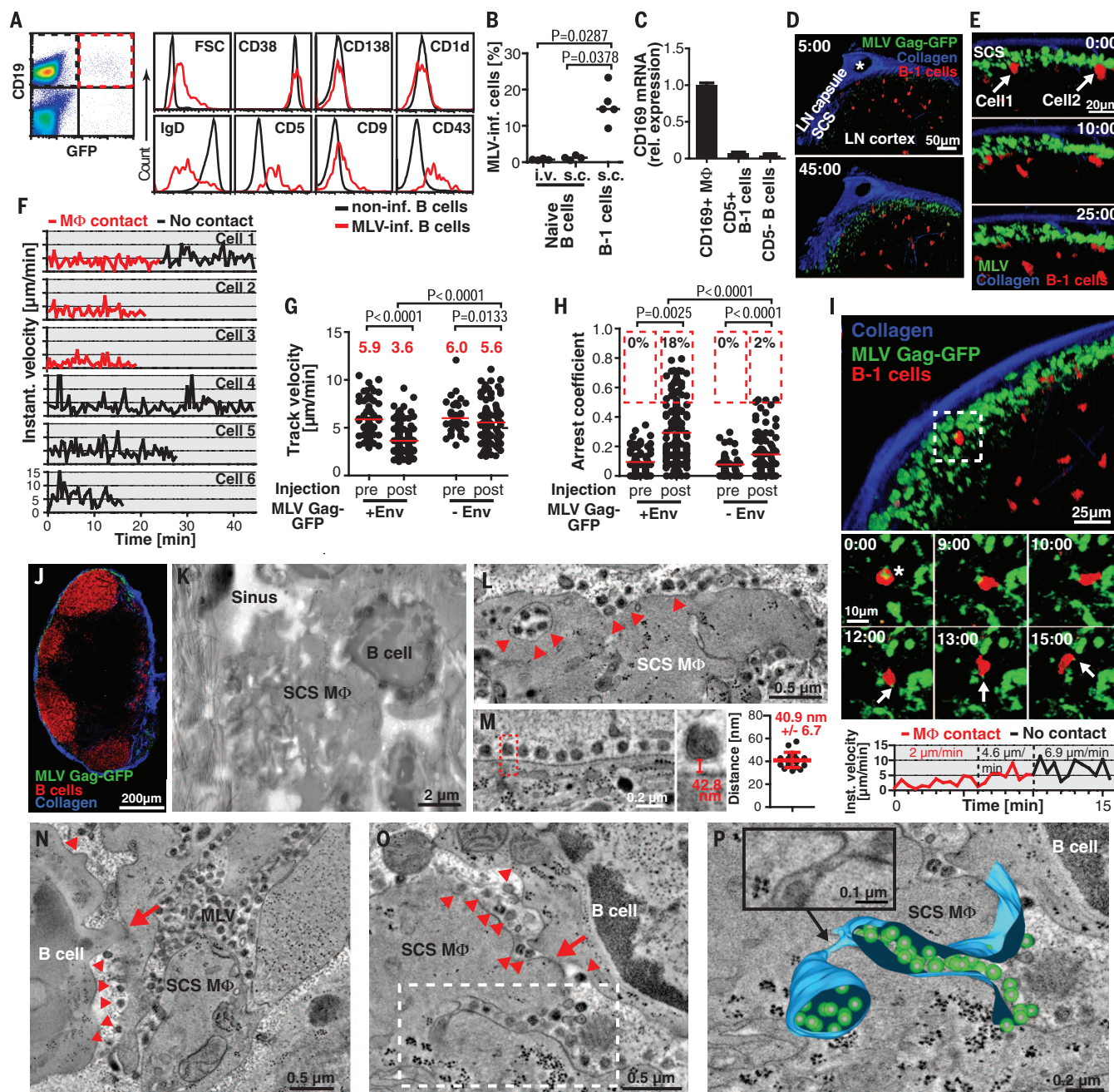


Fig. 2. MLV-laden CD169⁺ macrophages form Env-dependent contacts with B-1a cells for trans-infection. (A) Surface marker analysis of MLV-infected B cells in pLNs of C57BL/6 mice 2 days after s.c. infection. (B) Quantification of MLV-infected, adoptively transferred B-1 cells (s.c.) or naïve B cells (s.c., i.v.) ($n = 4$ to 5). (C) QPCR analysis of relative CD169 mRNA expression by pLN-derived CD5⁺ B-1a cells, CD5⁺ B cells, and CD169⁺ macrophages. Mean $\pm$ SD shown. (D) Images (movie S5) of MLV (green) capture at pLN SCS floor containing RFP⁺ B-1 cells (red). Asterisk depicts afferent lymphatic vessel entry site. Blue, collagen. Time in minutes. (E) Image sequence (movie S6) of adoptively transferred RFP⁺ B-1 cells (red) and captured MLV Gag-GFP (green) at the pLN SCS floor. Arrows depict cells analyzed in (F). Time in minutes. (F) Instantaneous velocity of representative B-1 cell traces (from Fig. 2E and fig. S15A) in contact with MLV-laden macrophages (red line) or not (black line). (G and H) Track velocities and arrest coefficients of adoptively transferred RFP⁺ B-1 cells in pLNs before and after s.c. injection of MLV Gag-GFP carrying or lacking Env (+/–Env). Red lines and numbers in (G) are medians. Percentages in (H) are cell population that remained arrested ($<2 \mu\text{m}/\text{min}$) $>50\%$ of time. Data are from four (+Env, 159 tracks) and

three (–Env, 138 tracks) independent experiments. (I) Image sequence (movie S8) of MLV Gag-GFP (green) transfer to RFP⁺ B-1 cell (red) at pLN SCS floor; and B-1 cell instantaneous velocity over time. Asterisk, stable contact ($t = 0$ min); arrows, MLV Gag-GFP transfer to B-1 cell uropod ($t = 12$ to 15 min). (J and K) Immunohistochemistry and electron microscopy overview of a pLN 1 hour after s.c. injection of MLV. (L and M) Electron tomography of a MLV-laden macrophage at the pLN SCS floor and quantification of the cell-virus distance ($n = 19$). Arrowheads depict MLV particles at the surface and within the macrophage. (N to P) Electron tomographies and tomographic 3D reconstruction of synaptic contacts between MLV-laden macrophages and B cells at pLN SCS floor 1 hour after s.c. injection of MLV. Arrowheads depict MLV particles at contact site. Arrows show direct cell-cell contacts between macrophages and B cells (N and O). In the studied tissue sections, we observe 2.14 contacts per 1000 μm^2 (mean, SD = 1.2; $n = 9$). Inset in (P) shows continuity between the invagination and a virus-containing compartment. Kruskal-Wallis test followed by Dunn's posttest for (B); Wilcoxon matched-pairs signed rank test for (G) and (H) (pre- versus postinjection); Mann-Whitney test for (G) and (H) (+Env_{post} versus –Env_{post}).

capture blood- or lymph-borne retroviruses in spleen and lymph nodes.

The capture of MLV and HIV by CD169⁺ macrophages could be the first step toward the initiation of host immune responses against incoming viruses. Alternatively, retroviruses may have evolved to use this pathway to efficiently infect their hosts. To investigate these possibilities, we monitored MLV infection following s.c. virus injection in wild-type C57BL/6 and *Siglec1*^{-/-} mice. MLV infection was significantly reduced in pLNs and spleen of *Siglec1*^{-/-} mice, indicating that virus capture by CD169⁺ macrophages contributes to efficient infection (Fig. 1, O and P). Despite reduced MLV infection in *Siglec1*^{-/-} mice, neutralizing antibodies were produced with similar kinetics and were able to control the spread of MLV infection by day 7, as in wild-type C57BL/6 mice (fig. S12). HIV infection of splenocytes in humanized mice was also significantly lowered upon CD169 blockade (Fig. 1Q). Collectively, these results demonstrate that retrovirus capture via recognition of gangliosides within the virus mem-

brane by CD169/Siglec-1 expressed on macrophages promotes efficient retroviral infection in vivo.

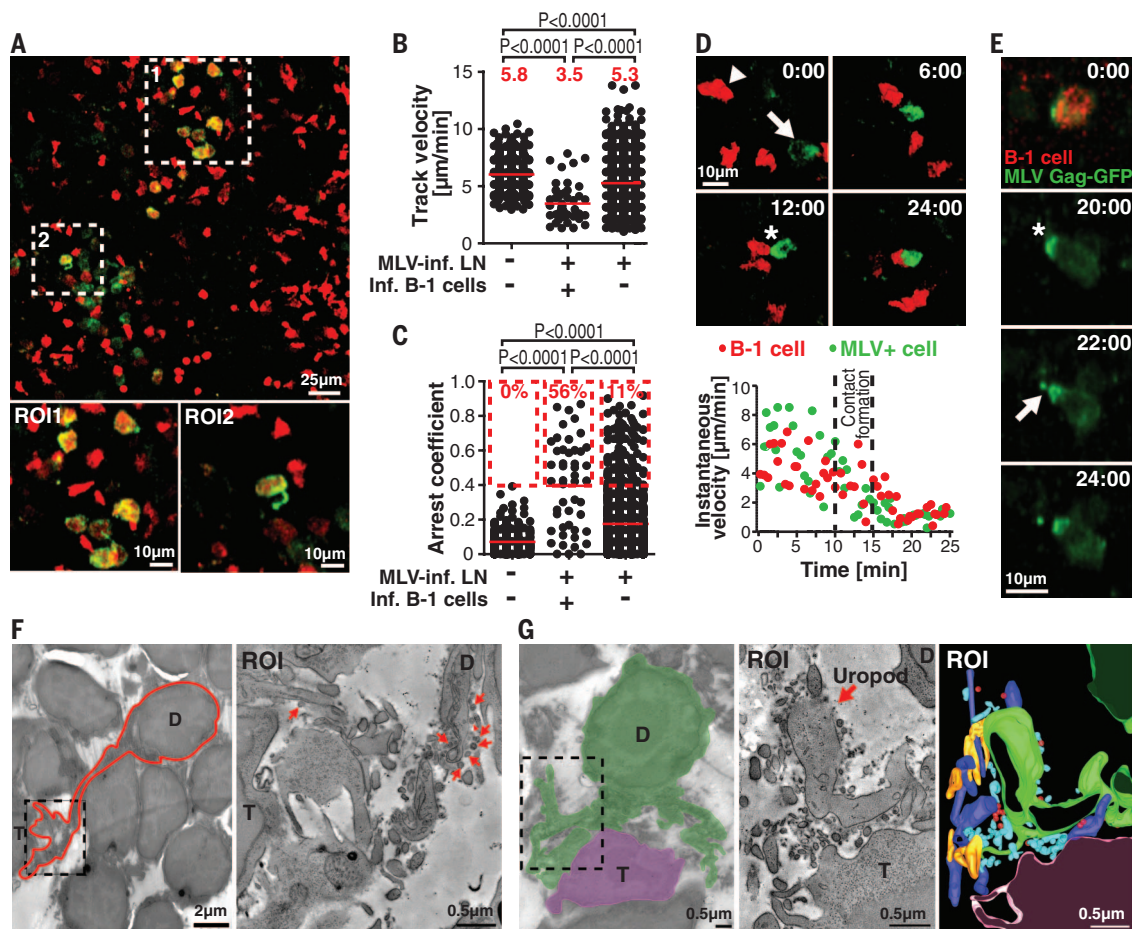
Interestingly, MLV does not infect CD169⁺ macrophages (19). Therefore, the likely role of CD169⁺ macrophages must be to trans-infect permissive lymphocytes. To visualize this, we first prepared naive B cells from mice expressing red fluorescent protein (RFP), adoptively transferred (i.v.) them into C57BL/6 mice, and infected the mice (s.c.) with MLV carrying a long-terminal repeat (LTR)-GFP reporter. Surprisingly, no RFP⁺ naive B cells were infected (fig. S13A and movie S4), yet MLV-infected cells were largely CD19⁺ B cells (19). We concluded that MLV must target a specific subset of B cells that is excluded during the preparation of naive B cells. We characterized these GFP⁺ MLV-infected cells as CD19⁺, CD43⁺, CD9⁺, CD5⁺, and IgD^{low}, which collectively defined them as B-1a cells (Fig. 2A) (20). B-1a cells are innate B cells, which secrete most of the circulating immunoglobulin M (IgM) (20). They undergo self-renewal

and thus are susceptible to MLV infection, owing to the virus's dependency on the cell cycle for nuclear entry (21). When we adoptively transferred (s.c.) RFP⁺ B-1 cells into C57BL/6 mice, incoming MLV specifically infected these cells but not naive B cells (Fig. 2B and fig. S13, B and C).

Having identified B-1a cells as target cells for MLV that lack CD169 expression (Fig. 2C and fig. S14), we attempted to directly visualize trans-infection events at the pLN SCS using two-photon laser scanning microscopy (2P-LSM). Upon arrival of MLV at the pLN, RFP⁺ B-1 cells were seen to sample the SCS, come in contact with MLV-laden SCS macrophages, and form long-lived synaptic contacts (Fig. 2, D to F; fig. S15A; and movies S5 to S7). B-1 cell migration velocity was significantly reduced upon contact with MLV-laden macrophages (Fig. 2F). This was also evident at the population level. B-1 cell track velocity decreased with a concomitant arrest coefficient increase after MLV injection (Fig. 2, G and H). In two instances (out of 159), following the disengagement from a synaptic contact with MLV-laden CD169⁺

Fig. 3. MLV-infected B-1 cells form stable virological synapses in infected pLNs.

(A) Image (from movie S11) of adoptively transferred RFP⁺ B-1 cells (red) in MLV Gag-GFP (green) infected pLN 2 days after infection. Regions of interest (ROIs) show Gag polarization (ROI1) and membranous protrusion (ROI2) of MLV-infected B-1 cells. (B and C) Track velocities and arrest coefficients of adoptively transferred RFP⁺ B-1 cells in noninfected and infected pLNs. Lines and numbers in (B) are medians. Percentage in (C) are static cell population that remained arrested (<2 $\mu\text{m}/\text{min}$) >60% of time. Data are from five independent experiments. Noninfected pLNs, 265 RFP⁺ B-1 cell tracks; MLV-infected pLNs, 48 MLV-infected and 361 noninfected B-1 cells tracks. Median is shown. Kruskal-Wallis test followed by Dunn's posttest. (D) Image sequence (movie S12) and instantaneous velocity of an adoptively transferred RFP⁺ B-1 cell (red, arrowhead) forming stable contact with MLV-infected leukocyte (green, arrow). Asterisk ($t = 12$ min) depicts contact formation. (E) Image sequence (movie S12) of MLV Gag-GFP material release (arrow) from an infected B-1 cell at a virological synapse (asterisk). Time in minutes. (F and G) Electron tomography and 3D reconstruction of pLN 2 days after s.c. infection, showing



MLV-containing membranous material at contact site between the uropod of MLV-infected donor cells (D) and uninfected target cells (T). Arrowheads indicate viral particles at surface of infected cell. In the studied tissue sections, we observe 2.6 membrane protrusion contacts per 1000 μm^2 (mean, SD = 1.1; $n = 11$). A 3D reconstruction [(G), right panel]: green, donor cell uropod; pink, target cell; blue and gold, uropod-associated membranous tubules; red, MLV.

macrophages, B-1 cells carried Gag-GFP-positive viral material at the uropod (Fig. 2I, fig. S15B, and movies S8 and S9). Though rare, these events are consistent with the notion that synaptic contacts contribute to virus transfer from SCS macrophages to B-1 cells. Mechanistically, synaptic contacts resulted largely from the interaction between Env on viral particles, presented on macrophages, and its receptor mCAT-1 expressed in B-1 cells (Fig. 2, G and H).

To understand trans-infection events at the ultrastructural level, we analyzed pLNs by electron tomography 1 hour after s.c. injection of MLV (Fig. 2, J to P, and fig. S16). Beneath a layer of collagen fibers, macrophages formed a dense layer at the SCS floor, followed by B cells at the distal side (Fig. 2, J and K). MLV particles were observed at the plasma membrane and within vesicle-like structures of SCS macrophages (Fig. 2L and fig. S16). Three-dimensional (3D) reconstruction revealed that these virus-containing compartments were continuous with the plasma membrane (Fig. 2, O and P; fig. S16; and movie S10). MLV particles bound to the macrophage surface with an average distance of 40.9 ± 6.7 nm (Fig. 2M), matching the predicted length of the CD169 molecule (22). We observed zones of contacts between macrophages and B cells with MLV virions in the cell-cell interface (Fig. 2, N and O, and fig. S16). A large invagination carrying dozens of viruses was found to open toward the B cell (Fig. 2P). These data suggest that retroviruses residing in deep plasma membrane invaginations in macrophages and DCs (23–25) can be mobilized toward contact zones and likely contribute to viral dissemination in vivo.

We followed the fate of MLV-infected B-1 cells over the next 2 days. After initially being trapped at the SCS, owing to interaction with MLV-presenting macrophages, MLV-infected B-1 cells redistributed deeper into the pLN to localize beneath the SCS, as well as in the interfollicular area and B-T cell border (fig. S13, D and E). They largely avoided B cell follicles and the T cell zone (fig. S13H). Uninfected peritoneal B-1 cells localized similarly at steady state (fig. S13F). The location of some MLV-infected cells to the T cell zone is explained, as ecotropic MLV also infects some CD4⁺ T cells (fig. S13G) (19). In contrast, amphotropic MLV specifically targeted the B-1a cell population (fig. S13I). Thus, despite using different receptors, both ecotropic and amphotropic MLV targeted the susceptible B-1a cell population.

At day 2, MLV-infected B-1 cells were often observed immobilized in foci with other uninfected cells, including B-1 cells (Fig. 3A movie S11). In addition to infecting adoptively transferred RFP⁺ B-1 cells, ecotropic MLV (GFP⁺) also infected endogenous B-1a cells and some T cells (Fig. 2A and fig. S13I) (19). At higher resolution, MLV Gag-GFP was seen to accumulate at the cell-cell interface between infected and uninfected cells (Fig. 3A and movie S11). These data indicate that MLV forms virological synapses during native infection (19). The immobilization of MLV-infected B-1 cells due to the formation of viro-

logical synapses was also evident at the population level. Compared to the behavior of uninfected B-1 cells, MLV-infected B-1 cells migrated at reduced velocities and exhibited higher arrest coefficients (Fig. 3, B and C). Because infected cells formed virological synapses with uninfected RFP⁺ B-1 cells, the latter also exhibited reduced migration (Fig. 3, B and C). Thus, as seen 2 hours after infection in the SCS (Fig. 2, G and H), the spread of MLV infection 2 days after infection can be seen at the population level simply by tracking B-1 cells. Our ability to visualize infected donor cells (MLV Gag-GFP⁺) and some target cells (uninfected RFP⁺ B-1 cells) allowed us to capture individual stages of the biogenesis of virological synapses (Fig. 3D, fig. S17, and movie S12). The transfer of Gag-GFP⁺ material from infected cells to uninfected cells (Fig. 3E and movie S12) indicated that these contacts can be associated with the transfer of viral material. Because we cannot detect the transfer of single particles by 2P-LSM in vivo, these transmission events must include clusters of particles, as has been observed for HIV in vitro (26). To gain insight into the ultrastructural information of virological synapses, we used electron tomography (27). We identified numerous complex membranous protrusions to which many viral particles were localized. These protrusions were often long and formed contacts between both neighboring and distally located cells (Fig. 3, F and G; fig. S18; and movies S13 and S14). These membrane-rich protrusions originated from a donor cell and were as long as ~ 10 μ m (Fig. 3F)—a structure that was also occasionally observed by intravital imaging (Fig. 3A ROI 2, movie S11). These protrusions were rich in intracellular vesicles and mitochondria, indicating that they represent uropods. We have previously observed that MLV assembles and buds at the uropod in polarized B cells in vitro (28). Similarly, HIV has been observed to assemble and bud at the uropod of polarized T cells (29). Our data suggest a model in which uropods form virological synapses in vivo to mediate transfer of viruses via large membranous protrusions.

Our data support a model in which trans-infection and virological synapses can both contribute to the spread of viral infections in vivo. The data are also consistent with a role for cell-free virus in spreading, as an alternative mode to virus spread via migration of infected cells (30). CD169⁺ macrophages are located at the interface between fluid phases such as the lymph, blood, and lymphoid tissue. They can concentrate cell-free viruses from the fluid phase to deliver them efficiently to permissive lymphocytes for infection. This model (fig. S19) suggests that viruses can disseminate by a combination of cell-free and cell-to-cell transmission, whereby viruses can use fluid flow-based dissemination over long distances and then cross the bottleneck at lymphoid tissue interfaces by exploiting the extraordinary ability of CD169⁺ macrophages to capture cell-free virus and trans-infect permissive lymphocytes. The relevance of this model for HIV transmission and its potential for therapeutic intervention will require further in vivo testing.

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SUPPLEMENTARY MATERIALS

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TUMOR VIRUSES

DNA tumor virus oncogenes antagonize the cGAS-STING DNA-sensing pathway

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Cyclic guanosine monophosphate–adenosine monophosphate synthase (cGAS) detects intracellular DNA and signals through the adapter protein STING to initiate the antiviral response to DNA viruses. Whether DNA viruses can prevent activation of the cGAS-STING pathway remains largely unknown. Here, we identify the oncogenes of the DNA tumor viruses, including E7 from human papillomavirus (HPV) and E1A from adenovirus, as potent and specific inhibitors of the cGAS-STING pathway. We show that the LXCXE motif of these oncoproteins, which is essential for blockade of the retinoblastoma tumor suppressor, is also important for antagonizing DNA sensing. E1A and E7 bind to STING, and silencing of these oncogenes in human tumor cells restores the cGAS-STING pathway. Our findings reveal a host-virus conflict that may have shaped the evolution of viral oncogenes.

Many viruses encode antagonists that enable evasion of innate immune detection of viral RNA and DNA (1). These antagonists can be broadly grouped into two classes. The first class consists of virus-encoded proteins that disrupt components of the

antiviral interferon (IFN) response that are shared among RNA and DNA sensors. Examples of this class include several herpesvirus-encoded proteins that block IFN signaling (2), and the Vpu accessory factor of human immunodeficiency virus (HIV), which degrades the interferon regulatory factor 3 (IRF3) transcription factor (3). A second class of virus-encoded antagonists targets the most proximal components of RNA and DNA sensing and thus mediates specific inhibition of one path-

way while leaving the other intact. For example, the nonstructural protein 3/4A (NS3/4A) protease of hepatitis C virus cleaves the mitochondrial antiviral-signaling (MAVS) adapter protein that is essential for RNA-activated innate immune signaling (4), and the NS1 protein of influenza A virus binds to and blocks activation of the retinoic acid-inducible gene I (RIG-I) RNA sensor (5).

We hypothesized that DNA viruses must encode dedicated antagonists of the proximal components of the DNA-sensing pathway that signal through cyclic guanosine monophosphate–adenosine monophosphate synthase (cGAS) (6) and the adapter protein STING/TMEM173 (7). Our exploration of cGAS-STING pathway antagonists began with our previous observation that most immortalized and tumor cell lines fail to respond to intracellular DNA, whereas primary cells mount a vigorous DNA-activated antiviral response (8). To explore this phenomenon in detail, we studied human embryonic kidney 293 (HEK 293) cells and HeLa cells, two of the most widely used human cell lines in biology. We transfected these cells with calf thymus (CT) DNA, a specific activator of the cGAS-STING pathway (9), or with a triphosphate RNA ligand that activates RIG-I (10). Whereas primary human fibroblasts mounted robust type I IFN responses to both DNA and RIG-I ligand (Fig. 1A), HEK 293 cells and HeLa cells responded only to RIG-I ligand and not to DNA (Fig. 1A). Similarly, primary mouse embryonic fibroblasts (MEFs) and bone marrow-derived macrophages responded to both DNA and RNA ligands, but immortalized mouse fibroblasts responded only to RNA (Fig. 1B).

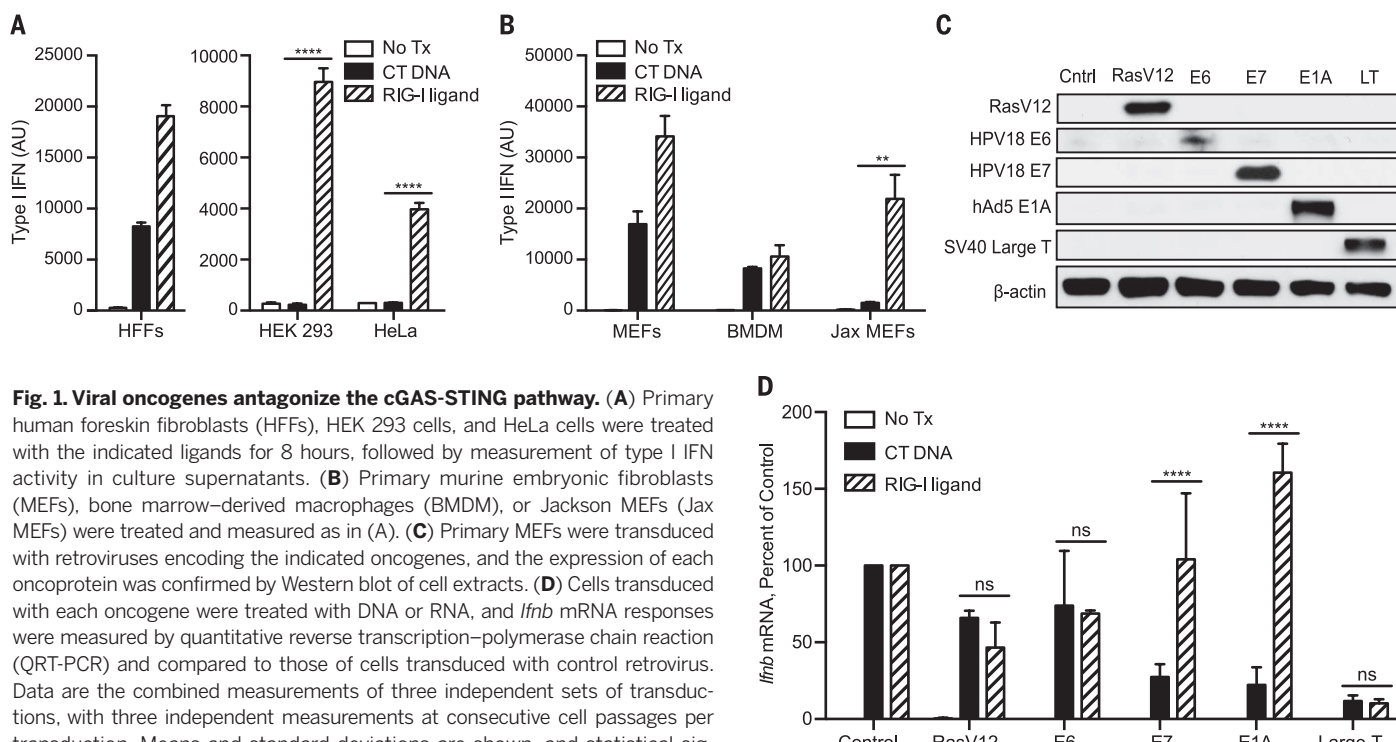


Fig. 1. Viral oncogenes antagonize the cGAS-STING pathway. (A) Primary human foreskin fibroblasts (HFFs), HEK 293 cells, and HeLa cells were treated with the indicated ligands for 8 hours, followed by measurement of type I IFN activity in culture supernatants. (B) Primary murine embryonic fibroblasts (MEFs), bone marrow-derived macrophages (BMDM), or Jackson MEFs (Jax MEFs) were treated and measured as in (A). (C) Primary MEFs were transduced with retroviruses encoding the indicated oncogenes, and the expression of each oncoprotein was confirmed by Western blot of cell extracts. (D) Cells transduced with each oncogene were treated with DNA or RNA, and *Ifnb* mRNA responses were measured by quantitative reverse transcription–polymerase chain reaction (QRT-PCR) and compared to those of cells transduced with control retrovirus. Data are the combined measurements of three independent sets of transductions, with three independent measurements at consecutive cell passages per transduction. Means and standard deviations are shown, and statistical significance was determined by using unpaired *t* test with equal SD with two-tailed *P*-values (A and B) or two-way analysis of variance (D): ***P* < 0.01; *****P* < 0.0001. ns, not significant.

These data demonstrate a specific loss of the cGAS-STING pathway in multiple immortalized cell lines.

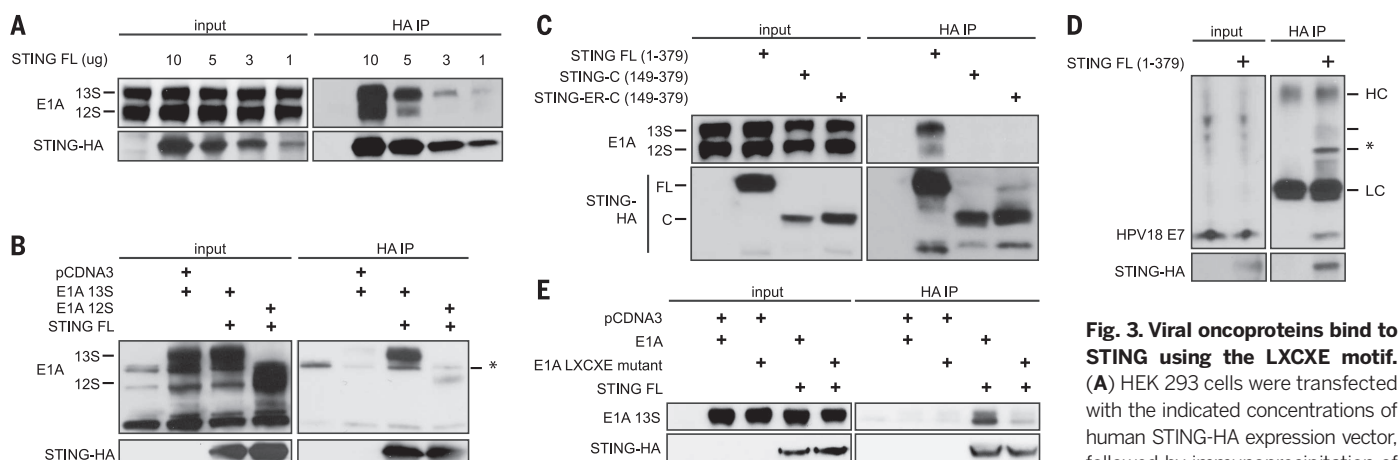
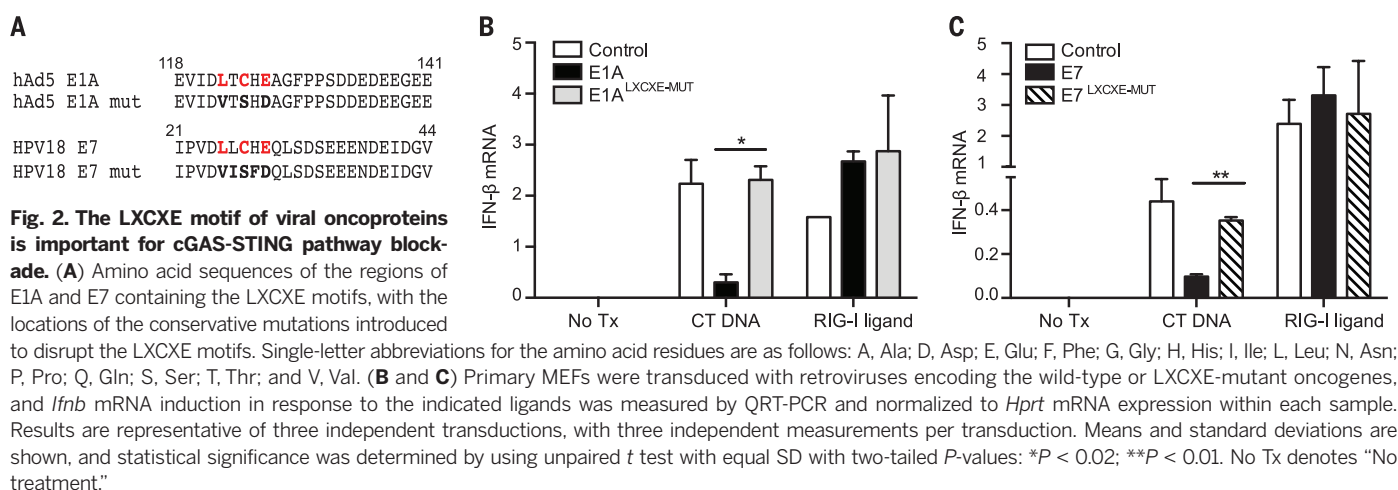
We considered the manner in which these cells were immortalized. HEK 293 cells were transformed by introduction of human adenovirus 5 (hAd5) DNA into fetal kidney cells (21). HeLa cells derive from a cervical carcinoma caused by infection with human papillomavirus 18 (HPV18) (12, 13). Our immortalized mouse fibroblasts were generated by introduction of the large T antigen of simian virus 40 (SV40). hAd5, HPV18, and SV40 are known as “DNA tumor viruses” (14), owing to their ability to cause cancer in experimental animals and (in the case of HPV18) in humans (15). These viruses encode dominant oncogenes that bind to and inactivate the two principal tumor suppressors in cells: p53 and retinoblastoma (Rb) (14, 16). Additionally, these viral oncogenes inter-

fere with IRF3- or IFN-dependent transcription that is shared by both the cGAS-STING and RIG-I-like receptor pathways, thus mediating generic inhibition of the antiviral response (17–20). Our findings led us to investigate whether these oncogenes might also specifically disrupt the unique proximal signaling components of the cGAS-STING pathway.

We generated retroviral expression vectors containing the oncogenes derived from these viruses: HPV18 E6, HPV18 E7, hAd5 E1A, and SV40 large T antigen. As a control, we included a constitutively activated form of the cellular proto-oncogene H-Ras (G12V) (21). We transduced primary MEFs with each of these retroviruses (Fig. 1C) and monitored transcription of IFN- β in response to transfection with DNA or RIG-I ligand. We found that transduction of MEFs with H-Ras G12V mildly affected their response to both RNA and DNA lig-

ands (Fig. 1D). Similarly, HPV18 E6 transduction resulted in a mild impairment of both pathways (Fig. 1D). Notably, transduction with HPV18 E7 or hAd5 E1A inhibited DNA-activated signaling, whereas the RIG-I pathway remained intact (Fig. 1D). Transduction with SV40 large T antigen led to a reduction in both cGAS-STING and RIG-I responses (Fig. 1D), suggesting that large T antigen may block a common component of the inducible antiviral response. These data reveal that E1A and E7 specifically block the cGAS-STING pathway when introduced into primary cells.

We next examined the mechanism by which E1A and E7 antagonize cGAS-STING pathway signaling. Both of these oncoproteins bind to and inhibit the function of the Rb tumor suppressor pathway, using a Leu-X-Cys-X-Glu (LXCXE) protein motif (22). To test whether the LXCXE



endogenous E1A protein or STING-HA. Inputs are shown in the left panels, and immunoprecipitations (IP) are in the right panels. (B) HEK 293 cells were transfected with 5 μ g of human STING-HA expression vector, together with either 13S E1A-FLAG or 12S E1A-FLAG, followed by immunoprecipitation of STING-HA and Western blotting for E1A-FLAG or STING-HA. (C) HEK 293 cells were transfected with the indicated STING-HA expression vectors and processed for immunoprecipitations and Western blot, as in (A). (D) HeLa cells were transfected with full-length (FL) STING-HA, followed by immunoprecipitation of STING-HA and Western blotting for endogenous E7 protein or STING. The asterisk indicates an unknown E7 antibody-immunoreactive protein migrating at ~35 kD. HC, immunoglobulin heavy chain; LC, immunoglobulin light chain. (E) HEK 293 cells were transfected with plasmids encoding wild-type or VXSXD-mutant E1A-FLAG, together with STING-HA expression vector, followed by immunoprecipitation of STING-HA. All results are representative of two (B) or three (A, C, D, E) independent experiments.

motif was also important for cGAS-STING pathway inhibition, we introduced conservative mutations into this motif (VXSXD) that are known to disrupt Rb binding (Fig. 2A) (23). We then transduced primary MEFs with retroviruses encoding these mutants and measured the responses of the transduced cells to DNA and RNA ligands. Whereas the wild-type E1A and E7 potently blocked the cGAS-STING response, the VXSXD mutants of these oncoproteins did not (Fig. 2, B and C). Neither the wild-type nor the mutant oncoproteins had an inhibitory effect on RIG-I-mediated signaling (Fig. 2, B and C). These data demonstrate that the mechanism of cGAS-STING pathway antagonism by E1A and E7 is similar to the mechanism by which they disrupt the Rb pathway.

On the basis of these data, we explored the cGAS-STING pathway-specific target of viral oncogenes. We first tested whether the three members of the Rb gene family (Rb, p107, p130) (24) were important for cGAS-STING pathway activation. However, primary mouse macrophages with lentiviral short hairpin RNA-mediated depletion of each of these Rb family members responded normally to DNA transfection (fig. S1, A and B). Moreover, triple-knockout MEFs lacking Rb, p107, and p130 similarly mounted a potent IFN- β response to DNA, despite their documented growth abnormalities (fig. S1C) (25). This demonstrated that the host target of viral oncogenes in the cGAS-STING pathway is distinct from their targets in cell cycle regulation. We therefore tested whether E1A and E7 targeted a proximal signaling component of the cGAS-STING pathway. We hypothesized that, similar to the means by which E1A and E7 disrupt Rb, these oncoproteins would block the cGAS-STING pathway through direct binding to a cellular protein. We did not detect an interaction between viral oncoproteins and cGAS. Notably, when we immunoprecipitated epitope-tagged human STING from

HEK 293 cells, we observed a robust interaction between STING and hAd5 E1A that is endogenously expressed in these cells (Fig. 3A). Moreover, we noticed that 13S E1A interacted more robustly with STING compared to 12S E1A, especially at lower concentrations of STING expression vector (Fig. 3A). Indeed, a direct comparison of STING binding to FLAG epitope-tagged 13S E1A or 12S E1A revealed a specific interaction with 13S E1A and a much weaker interaction with 12S E1A (Fig. 3B), demonstrating that the unique zinc finger motif present in 13S E1A is important for robust STING binding.

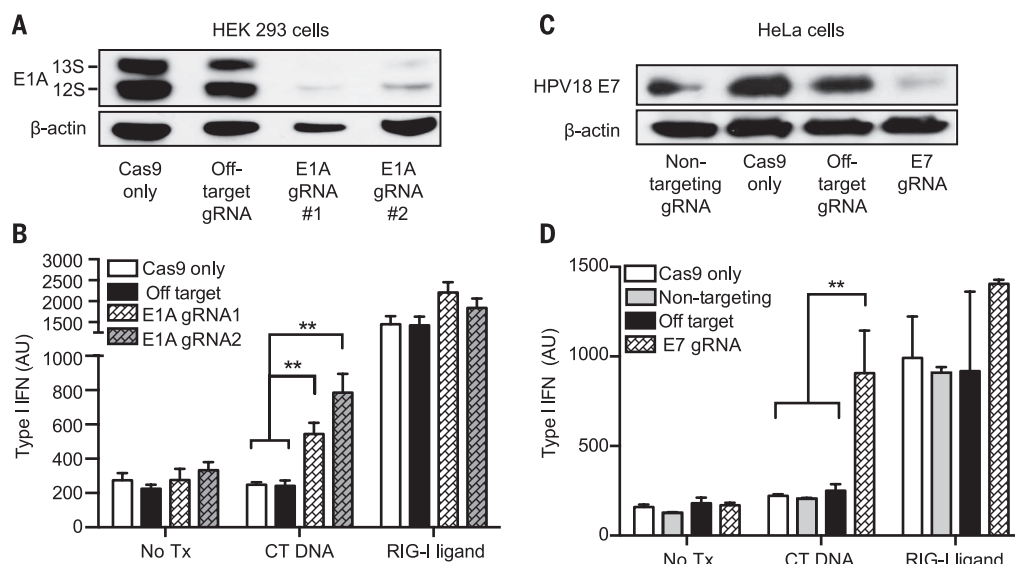
We next tested whether specific domains of STING were important for interaction with E1A. The N-terminal 148 amino acids of STING constitute the transmembrane domains that anchor it to the endoplasmic reticulum (ER) membrane, whereas amino acids 149 to 379 encode a cytosolic signaling domain that mediates STING dimerization and binding to cyclic dinucleotides and IRF3 (26, 27). We were unable to express the N terminus of STING to appreciable levels in HEK 293 cells, suggesting instability of this domain in live cells. However, using epitope-tagged constructs encoding STING amino acids 149 to 379, or 149 to 379 anchored to the ER membrane by a heterologous transmembrane domain derived from cytochrome p450 (28), we found that the isolated C terminus of STING is insufficient to mediate interaction with E1A (Fig. 3C), revealing that additional elements of STING are required for robust interaction with E1A. We then overexpressed STING in HeLa cells and detected an interaction with the endogenous HPV18 E7 protein (Fig. 3D). We reproducibly detected a ~35-kD protein with the E7 antibody that also specifically interacted with STING (Fig. 3D, asterisk), the nature of which is unknown.

We examined whether the LXCXE motif in E1A that is essential for blockade of the cGAS-STING

pathway (Fig. 2) is also important for E1A binding to STING. To do this, we generated FLAG epitope-tagged 13S E1A constructs containing either the native LXCXE motif or the mutant VXSXD motif. We found that the VXSXD mutant of E1A was severely compromised for interaction with STING-hemagglutinin A (HA) in HEK 293 cells (Fig. 3E). Together, these data identify STING as a binding partner of E1A and E7 and suggest a potential mechanism of cGAS-STING pathway blockade by viral oncogenes. The contribution of the LXCXE motif of E1A to STING binding precisely mirrors the role of this same motif in Rb binding, revealing a mechanistic parallel between antagonism of the DNA-activated antiviral response and inactivation of a tumor suppressor.

We returned to the transformed human cells that are specifically unresponsive to transfection with DNA. We hypothesized that the constitutive expression of E1A (HEK 293) or E7 (HeLa) renders these cells unable to activate the cGAS-STING pathway. To test this, we used a lenti-CRISPR (clustered regularly interspaced short palindromic repeat) approach (29) to disrupt these oncogenes. Notably, we found that the E1A-targeted HEK 293 cells were able to mount an IFN response to DNA transfection, but cells transduced with control guide RNAs remained as unresponsive as the parental cells (Fig. 4, A and B). We next used the same lenti-CRISPR approach to target the ~12 copies of E7 in HeLa cells (Fig. 4C), where we also observed robust recovery of the cGAS-STING pathway (Fig. 4D). Moreover, we found that HeLa cells transduced with a retrovirus encoding the bovine papillomavirus E2 protein, which silences the promoter encoding the bicistronic HPV18 E6/E7 mRNA (30), also recovered a potent IFN response to DNA transfection (fig. S2). Together, our data demonstrate that HEK 293 cells and HeLa cells both retain a functional cGAS-STING pathway, but this pathway is inhibited by the

Fig. 4. Restoration of the cGAS-STING pathway in human tumor cells. (A and B) HEK 293 cells were transduced with lenti-CRISPR constructs containing the indicated guide RNAs (gRNA), selected for 3 days in puromycin, and then harvested for evaluation of E1A protein expression by Western blot (A), or for plating prior to stimulation with the indicated ligands (B). Type I IFN activity was measured in culture supernatants 24 hours after stimulation. (C and D) HeLa cells were transduced with Lenti-CRISPR constructs containing the indicated gRNAs, selected for 3 days in puromycin, and then harvested for evaluation of E7 protein expression by Western blot (C), or for plating prior to stimulation with the indicated ligands (D). Type I IFN activity was measured in culture supernatants 24 hours after stimulation. Data are representative of three independent experiments. Means and standard deviations are shown, and statistical significance was determined by using unpaired *t* test with equal SD with two-tailed *P*-values: ***P* < 0.01.



constitutive expression of the viral oncogenes that transformed these cells.

Our data reveal that DNA tumor virus oncoproteins are potent and specific antagonists of the DNA-activated antiviral response. Recently, an unrelated herpesvirus-encoded protein was also found to bind to STING and block its activation (31), suggesting that targeting of STING may represent a general mechanism by which DNA viruses manipulate innate immune signaling. Notable advances over the last four decades have revealed how viral oncogenes cause cancer through inhibition of p53 and Rb (14, 16, 24). Our findings may shed new light on the long-standing question of why DNA tumor viruses evolved these oncogenes in the first place. There are two hypotheses that rationalize the origins of these oncogenes, neither of which is mutually exclusive. The “limited resources hypothesis” (16) postulates that oncogenes evolved to stimulate cell cycle progression in order to facilitate a cellular environment conducive to viral replication: abundant deoxynucleoside triphosphates for viral DNA synthesis, dissolution of the nuclear envelope to allow for viral egress, and new cellular niches to support transmission. In contrast, the “anti-antivirus hypothesis” (14) holds that the principal function of viral oncogenes is to antagonize innate immune signaling. Our data support the latter of these two hypotheses and reveal a host-virus conflict that may have shaped the origins of the DNA viruses that cause cancer in humans. Interestingly, most DNA viruses that infect humans encode proteins with

LXCXE motifs (32), but only a minority of these viruses are known to cause cancer. We propose that study of these LXCXE-containing proteins will illuminate a broad class of STING-pathway antagonists.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/350/6260/568/suppl/DC1
Materials and Methods
Figs. S1 and S2

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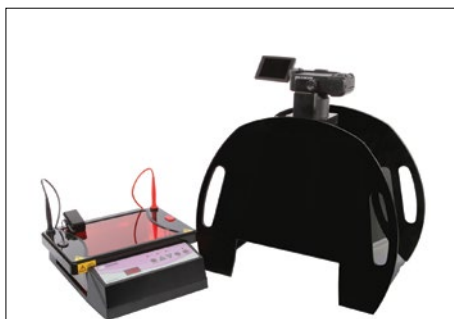
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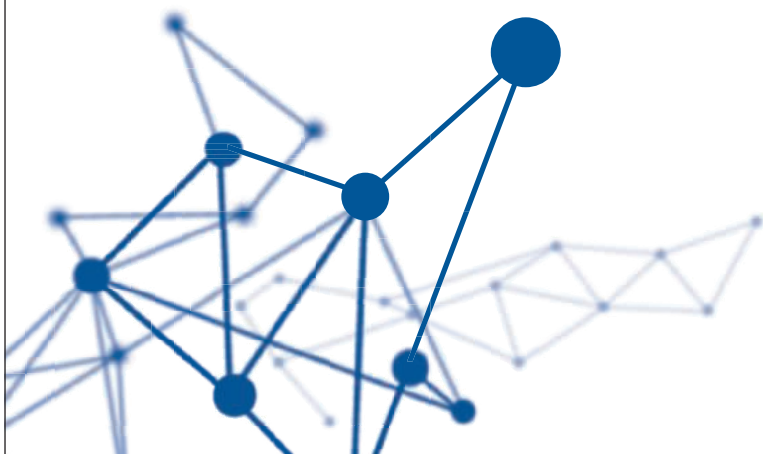
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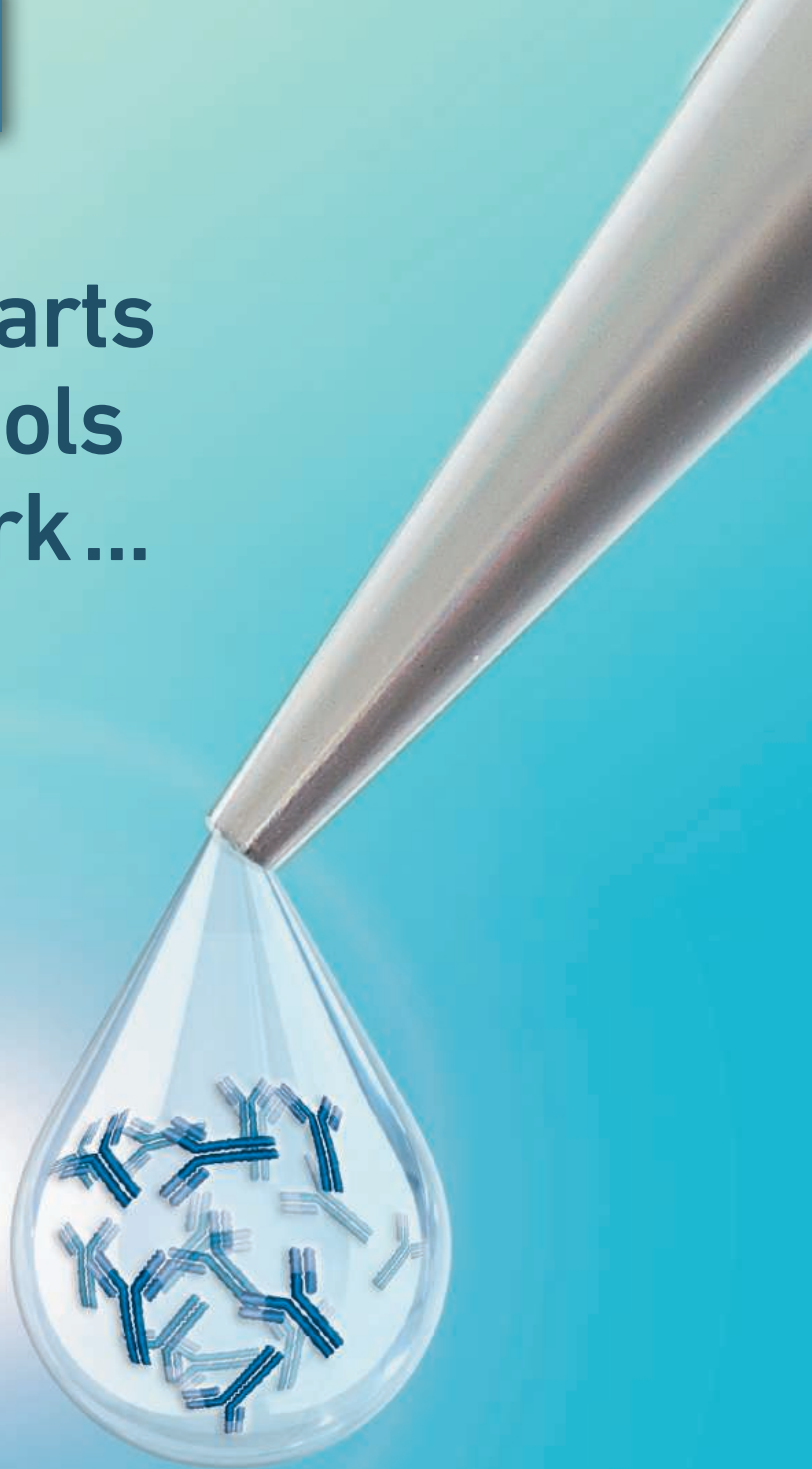
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FROM THE JOURNAL SCIENCE AAAS



Top firms prioritize transformative technologies, patients



The biotechnology and pharmaceutical sectors have emerged from the global recession to find technological breakthroughs driving renewed enthusiasm and risk-taking at companies. The firms landing at the top of the 2015 *Science* Careers Top Employers Survey have harnessed these innovative sparks and created workplaces that recruit the best and brightest scientific minds. Their researchers are largely given free rein to develop the next big thing in green agricultural biotechnology (agbiotech) or cancer treatment. Real-world results from such breakthrough innovations inspire the next generation of industry researchers as they ride the crest of a new wave of biological advances. These top employers ensure that their scientists surf that wave with agility, passion for what they do, and creativity to arrive at technologies that will transform lives. **By Kendall Powell**

It sounds like an old game show. The buzzword is “transformative technologies.” But it’s truly more than a buzzword. A palpable excitement travels through both boardrooms and scientific conferences following breakthroughs in immunotherapy, messenger RNA (mRNA) therapies, or microbiome mining. Many companies have made a strategic migration away from the tried-and-true (but also too long and costly) pathways of drug discovery toward novel approaches that promise unprecedented speed and precision.

That excitement is one reason that scores in this year’s *Science* Careers Top Employers Survey were higher in general—more so than in the last four years. A better economic outlook, with venture capital money flowing into the biotechnology and biopharmaceutical sectors more freely, doesn’t hurt either. The scores reflect a breath of fresh air from scientific advances that translate into not merely incremental advances, but rather transformative new medicines or solutions.

This year like all others, scientists want to work at companies that keep innovation front and center, and the top 20 employers in 2015 include those biotechnology and pharmaceutical firms on the leading edge of these advances. Three new players arrive on the top 10 leaderboard this year, focused on making the world a greener place, developing therapies for unique patient populations, or creating a revolutionary technology platform—all first-time survey participants. Each firm is relatively small, but with growing global reach: Novozymes (#1) has 6,000 employees, Alexion Pharmaceuticals (#5) has 2,800 employees, and Moderna Therapeutics (#7) has more than 250 employees.

“We’re a mid-sized company and that actually matters,” says **Peder Holk Nielsen**, chief executive officer of top-ranked

Novozymes. “It’s possible to maintain a family-like environment and still operate globally.” Novozymes boasts of an uber-friendly workplace (rooted in the company’s Danish heritage) and a culture that is science-centric. Twenty-one percent of the company’s employees are working in labs, and the company touts the fact that 14% of its total revenue is reinvested into R&D annually.

“Zymers,” as company employees are called, are also given high levels of responsibility from day one. “It means that young scientists will be charged with significant programs without a lot of managerial follow-up,” says Nielsen. “We trust people to do their best—it’s a flat organization where everyone can talk to everyone else—and you are expected to have an opinion and voice it.” Politely, of course.

All the top employers included here exhibit these notions of open communication, flat hierarchies, and environments that encourage scientists to challenge conventional wisdom. As the highest-revenue pharmaceutical company to make the top 10, Roche (#8) had 17,566 R&D employees and invested \$9 billion in R&D in 2014. Bristol-Myers Squibb (BMS), which returns to the survey at #20 after an eight-year absence, has a corporate stance of taking big risks while following solid science. Biogen (#13), based in Bengaluru, India, returns to the list this year as the only firm headquartered in Asia. And Celgene Corporation (moving up to #12 this year after placing #17 in 2013 and #15 in 2014) leapt ahead of other mid-sized biopharma peers, Biogen (#18) and Gilead (#19).

These successful workplaces keep creativity and innovation at the heart of operations—giving employees the responsibility and control over developing projects and their own careers. These firms are filled with motivated employees because real-life examples of their work’s impact on the world are woven into their cultures. And though recruiting and retaining a highly skilled work force is cited as a major challenge for the industry, these companies excel at attracting and keeping top talent. Benefits, both official and fun perks, keep employees’ eyes on the prize—developing bold ideas into the next transformative application.

“We are not looking for therapies that give incremental benefits,” says **Martin Mackay**, head of research and development for Cheshire, Connecticut-based Alexion. He explains that a focus on rare and ultrarare diseases grew organically out of the company’s mission to find transformative therapies for devastating diseases. One such condition, hypophosphatasia (HPP), can be fatal in severe cases in newborns who lack a properly formed ribcage needed for breathing. This year, Alexion anticipates U.S. approval for Strensiq, an enzyme replacement therapy for HPP. **continued >**

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Top twenty employers



The 20 companies with the best reputations as employers and the top three driving characteristics for each company, according to respondents in the 2015 survey undertaken for the Science/AAAS Custom Publishing Office. The companies without a 2014 rank did not receive enough mentions to qualify or did not receive a high enough ranking during the 2014 survey.

2015 Rank	2014 Rank	Employer (Global headquarters)	Innovative leader in the industry	Treats employees with respect	Has loyal employees	Is socially responsible	Work culture values aligned
1	–	Novozymes (Bagsvaerd, Denmark)	✓	✓		✓	
2	1	Regeneron Pharmaceuticals, Inc. (Tarrytown, NY)	✓	✓	✓		
3	2	Novo Nordisk (Bagsvaerd, Denmark)	✓		✓	✓	
4	4	Vertex Pharmaceuticals (Boston, MA)	✓			✓	✓
5	–	Alexion Pharmaceuticals (Cheshire, CT)	✓		✓	✓	
6	3	Genentech (South San Francisco, CA)	✓	✓			✓
7	–	Moderna Therapeutics (Cambridge, MA)	✓	✓	✓		
8	8	Roche—excluding Genentech (Basel, Switzerland)	✓		✓	✓	
9	6	Monsanto Company (Creve Coeur, MO)	✓	✓	✓		
10	7	AbbVie (North Chicago, IL)			✓	✓	✓
11	5	Eli Lilly and Company (Indianapolis, IN)		✓	✓	✓	
12	15	Celgene Corporation (Summit, NJ)	✓	✓	✓		
13	–	Biocon (Bangalore, India)	✓		✓	✓	
14	12	Abbott (Abbott Park, IL)			✓	✓	✓
15	13	Boehringer Ingelheim (Ingelheim, Germany)		✓	✓	✓	
16	16	Bayer (Leverkusen, Germany)		✓		✓	✓
17	20	Merck KGaA (Darmstadt, Germany)			✓	✓	✓
18	10	Biogen (Cambridge, MA)	✓			✓	✓
19	–	Gilead (Foster City, CA)	✓	✓	✓		
20	–	Bristol-Myers Squibb (New York, NY)	✓	✓	✓		

Mackay ticks off the impressive results that patients and their families have experienced: “Babies breathing on their own, children growing at normal rates, even playing sports—this is the daily drive we have at Alexion.”

What makes top employers shine?

Each year, *Science Careers* commissions a survey to identify the top employers in the biotechnology and pharmaceutical industry and to determine the characteristics that best describe a top employer. This year, the results are based on 5,700 responses to a web-based survey deployed by e-mail (see Survey Methodology online at www.sciencecareers.org/topemployers2015).

The vast majority of respondents are scientists working in areas of basic or applied research and development (see Survey Demographics box, page 578). Of the one-fifth of respondents likely to seek a new job in the next year, more than half will do so to seek career advancement or new opportunities. Human resources officers at top firms are not surprised—they say employees place a higher emphasis on career development than total compensation.

In selecting the best companies, respondents yet again chose “innovative leader” as the top-driving characteristic. A top employer is also defined as an organization that “treats employees

with respect,” “has loyal employees,” “is socially responsible,” and has a “work culture aligned” with employees’ values (see Driving Characteristics table, 580).

The 2015 survey included a way for respondents to rank the biggest advantages to working in the biopharma industry. Workers voted “innovation” solidly as #1, followed by “working with smart colleagues” and “excellent compensation and benefits” as a close #2 and #3, respectively. Interestingly, workers ranked having the funding and resources for research projects as a more distant #4.

Companies meeting those challenges adeptly and fulfilling those advantages include Regeneron Pharmaceuticals, Incorporated (#2), Novo Nordisk (#3), Vertex Pharmaceuticals (#4), Genentech (a member of the Roche group) (#6), Monsanto Company (#9), and AbbVie (#10), which round out the top 10 employers (see chart above for full top 20 list).

The comeback company

BMS’s comeback as a top employer after last appearing on the list in 2007 is no mere coincidence. That period aligns with a company-wide makeover, during which BMS made a series of decisions to turn around a firm that had become one of the least profitable in the industry. *continued*

The background of the advertisement features a photograph of two scientists in a laboratory. In the foreground, a woman with dark hair tied back, wearing safety glasses and a white lab coat, is focused on a piece of scientific equipment. In the background, a man with glasses and a white lab coat is also working. The overall atmosphere is professional and scientific.

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Demographics

Gender:

54% Male, 42% Female, 4% No response

Experience:

65% have 10 or more years work experience

Highest degree earned:

37% Doctorate, 32% Master's, 24% Bachelor's, 7% Other

Company type:

34% Pharma, 34% Biotech, 24% Biopharma, 2% University, 6% Other; More than 9 out of 10 work in private industry

Nature of work:

36% Development, 28% Applied Research, 20% Basic Research, 10% Administration/Executive, 14% QA/QC/Regulatory Affairs, 8% Production, 11% Other (respondents were able to choose more than one response)

Geography:

57% from North America, 27% from Europe, 11% from Asia/Pacific Rim, 5% from rest of world

The firm dropped discovery and development in the areas of diabetes, virology, and neuroscience in favor of a research focus on oncology, heart failure, genetically defined diseases, immunoscience, and fibrotic diseases. In 2009, BMS took a big risk jumping into the immunotherapy field by acquiring the company Medarex, which brought with it Yervoy, a member of a new class of cancer drugs. In 2011, Yervoy became a breakthrough treatment for metastatic melanoma, and BMS quickly followed that success with another immuno-oncology drug, Opdivo.

By June 2015, the transformation was largely complete, with BMS renewing its focus on the abovementioned areas and also on immuno-oncology, where it held a huge lead over the competition. The company that emerged on the other side was slimmer, shedding the weight of a large, too-diversified drug company to become a biopharmaceutical firm focused on specialty therapies for high medical needs.

"It completely changed the atmosphere. It's changing the outcomes for these terrifying diseases," says **Fouad Namouni**, head of development for Yervoy and Opdivo at BMS's headquarters in Princeton, New Jersey. "We are walking the talk, trying to help save lives." As such, he says the makeover was not only the right business decision, but was highly motivating for BMS researchers, too. BMS had 7,300 R&D employees, invested \$4.5 billion in R&D in 2014, and expects to open a new R&D campus in Cambridge, Massachusetts in 2018.

Namouni credits trailblazing and bold leadership for BMS's success in immuno-oncology. "No matter what the science was telling us, we followed it and applied it, even during times when people thought it was heresy."

Riding the immunotherapy wave

And people did think so. Although the idea of immunotherapy—activating or boosting the body's natural defenses to fight diseases better—has been around for decades, the molecular keys to unleash the immune system without doing more damage than good have long remained mysterious. But in the last decade, the field has made a tsunami of progress. Now, pharmaceutical manufacturers are jostling

for position on the immunotherapy wave, with BMS holding the leader's position.

BMS's two monoclonal antibody products both act on T-cell checkpoints, mechanisms that normally act to shut down an immune response once the job is finished. Some cancers have also found ways to trigger these checkpoints to effectively shut off T cells and hide from the immune system. By masking checkpoint receptors, Yervoy and Opdivo expand the number of circulating, tumor-recognizing T cells.

"The consequence is that the T lymphocytes are back at work and our natural defense system does a very good job" attacking tumors, says Namouni. Both drugs are approved for treating metastatic melanoma, and Opdivo is also approved for squamous lung cancer. Namouni says the immuno-oncology field exploded after BMS showed that the immune-activating approach not only worked, but worked on notoriously stubborn cancers.

These amazing successes have made believers of investors and researchers alike, with a flood of companies adding cancer immunotherapy components to their portfolios. Both Celgene and Roche have firmly staked out territory on the immunotherapy stage already.

Roche has four biologic cancer immunotherapy molecules in clinical trials that could work in powerful combinations with each other or with current drugs, says **William Pao**, global head of oncology discovery in Basel. Those candidates include antibodies that would activate and arm more T cells and bispecific, engineered antibodies that physically bring T cells to the tumor cells they are armed to kill. Another engineered antibody would tag tumor cells with an immunocytokine that preferentially activates killer T cells.

Much like BMS, Celgene also made a big gamble about a decade ago when it developed a class of immune-modulating drugs that included the infamous teratogen thalidomide. These immunomodulatory drug (IMiD) compounds, including Revlimid, were successful at targeting multiple myeloma and lymphoma. They work by boosting the degradation of key factors for white blood cell production.

Transformative biotechnologies

Other top employer innovations harbor the potential to change lives as well. Transformative biotechnologies at Moderna and Novozymes are changing the way scientists approach both medicine and agriculture.

Novozymes is a relative newcomer to the biopharmaceutical realm, having split from Novo Nordisk in 2000. Headquartered in Bagsvaerd, Denmark, the enzyme-based company makes industrial, biofuel, agricultural, and medical products. Some of Novozymes' latest technological pushes rely on mining the microbiome to find powerful new enzymes or activities.

Chief Scientific Officer **Per Falholt** says that the enzymes discovered to date are only the tip of the iceberg. "In the past, we were restricted to microbes we could grow in the lab, but metagenomics gets around that," he says.

Teaming up with fellow top employer Monsanto, Novozymes' scientists are developing microbial seed treatments that will yield more corn and soybeans, ideally with less chemical fertilizers, pesticides, or water. These microbes might increase crop yields by releasing more phosphate or nitrogen from the soil. **Nathan Cude** works in Novozymes' agbiotech division in Durham, North Carolina in the microbial discovery group, which isolates and identifies thousands of microbes collected from soil samples around the United States. After characterizing the bugs genetically and biochemically, and assessing safety risks, the group nominates promising candidates to Monsanto for testing in 500,000 annual field trials of every imaginable soil and weather scenario. **continued>**



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Driving characteristics of top employers

2015:	2014:
1. Innovative leader in the industry	1. Innovative leader in the industry
2. Treats employees with respect	2. Treats employees with respect
3. Loyal employees	3. Loyal employees
4. Socially responsible	4. Socially responsible
5. Work culture values aligned	5. Work culture values aligned
	6. Makes changes needed

Driving characteristics are listed in descending order of impact on overall employer rankings.

The colored backgrounds indicate the characteristics in common for the two years.

Moderna's mRNA therapeutics also put the power of molecular genetics to work, but, in this case, as an entirely new drug modality. Formed in 2011 and based in Cambridge, Massachusetts, Moderna is the newest top employer on the block. The company's modified mRNA drugs incorporate naturally occurring nucleotide analogs that evade the body's efficient dispatch of foreign, introduced RNA.

Matt Stanton, Moderna's head of chemistry, says the company's innovation can use exogenous, synthesized mRNA to create any protein of interest in targeted cell types or tissues. "There are obvious no-brainer advantages to that approach" in cost, speed, and efficacy, he says.

Moderna Chief Executive Officer **Stéphane Bancel** adds: "mRNA drugs can do things for patients that small molecules and huge antibodies cannot do." Among other feats, the technology has the potential to serve up gene therapy without genetic tinkering, and it can deliver regenerative medicine without the messiness of cell-based therapies. Stanton says that the technology could also tackle "undruggable" targets, for example by replacing a missing intracellular protein or disrupting a protein-protein interaction.

Innovation has remained the survey's top driver for 12 years running. It's not surprising that scientists want employers who give them the space and freedom for the creativity needed to find to fresh solutions. Celgene scientist **Patrick Hagner** develops next-generation IMiD therapies, including drug candidate CC122. When asked what he likes best about working there, he replies, "The nerd in me says innovative science is what defines this company."

But he also mentions a particular 20-year-old lymphoma patient whose cancer had failed to respond to multiple chemotherapies. After enrolling in a clinical trial for CC122, the patient experienced a remission. "To have actually helped somebody live longer—that's one of the most enjoyable qualities of working here."

Putting patients, planet first

Hagner is not alone in being motivated by making such a tangible difference. Most top employers scored highly for being responsible corporate citizens and for having corporate values that aligned with their employees' own beliefs. Many have sustainability initiatives like buildings powered by wind and recycled water (Celgene), partnering with local Habitat for Humanity projects (Moderna), or volunteering at patient events like the National Veterans Wheelchair Games (BMS). Last year, Celgene employees raised funds alongside the Multiple Myeloma Research Foundation in the Empire State Building Run-Up, racing up all 86 flights of stairs in less than 15 minutes.

Two firms, however, stand out in the crowd for placing patients' needs (Alexion) and sustainability (Novozymes) squarely at the center of their business model. "In this industry, everyone is trying

to come up with important, good medicines, but at Alexion, we are extremely and genuinely patient-centric," says Mackay.

That "patientricity" can be seen in town-hall meetings where patients share their disease and treatment experiences. After visits from Alexion's youngest patients, Mackay says he often sees employees "walking on air, going back to their lab benches or offices knowing that they could have a real impact on children."

The urgency to find treatments for life-threatening conditions translates into a company culture that is fast-paced, hardworking, and entrepreneurial in spirit, says **Clare Carmichael**, chief human resources officer for Alexion.

Similarly, Novozymes' Nielsen says that young employees are not driven by the size of their paychecks, but rather by personal development and making an impact. "People want to tell their kids when they pick them up from kindergarten, 'I did something today that makes this world a better place to live,'" he says.

The company's tagline "make more with less" plays out across its science, from detergent enzymes that save energy and water to technology for converting waste biomass into biofuels. It even trickles into travel planning, with employee reminders about carbon footprints.

That emphasis on sustainability appealed to scientist **Leah Blasiak** when she transitioned from academia to her current post in the agbiotech division at Novozymes. "What I do matters, and I am much closer to the direct application of my research," she says.

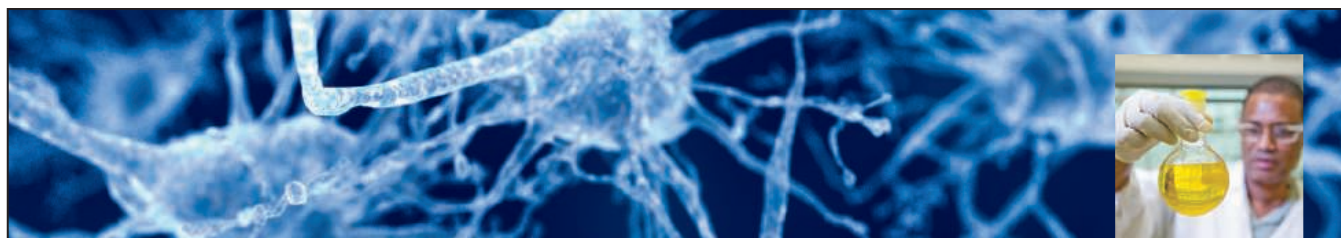
Recruiting and retaining top talent

Recruiting talented young scientists like Blasiak and keeping them on board for the long haul was cited by this year's survey respondents as one of the industry's biggest challenges. Top firms say they have not-so-secret weapons for attracting the best scientists and keeping them stimulated.

"Novozymes' success is determined by the passion and energy that Zymers bring to work each morning," says Nielsen. He says his firm is often a first choice for scientists in Denmark, Sweden, and Germany who are familiar with it, but recruiting in the United States or Asia is more difficult.

Falholt says that Novozymes looks for scientists who "burn high," chewing on problems until a solution comes to them, whether during work hours or not. Likewise, every Moderna employee is given an iPhone and iPad connected to the company cloud, so if genius strikes while an employee reads her Sunday paper, it can be captured instantly.

Many top employers are growing rapidly, and so they look for employees who are "learning agile," who can wear multiple, shifting hats, and who excel at cross-functional or even cross-company collaboration. Job candidates must **continued>**



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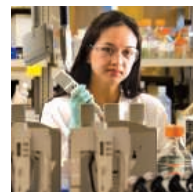
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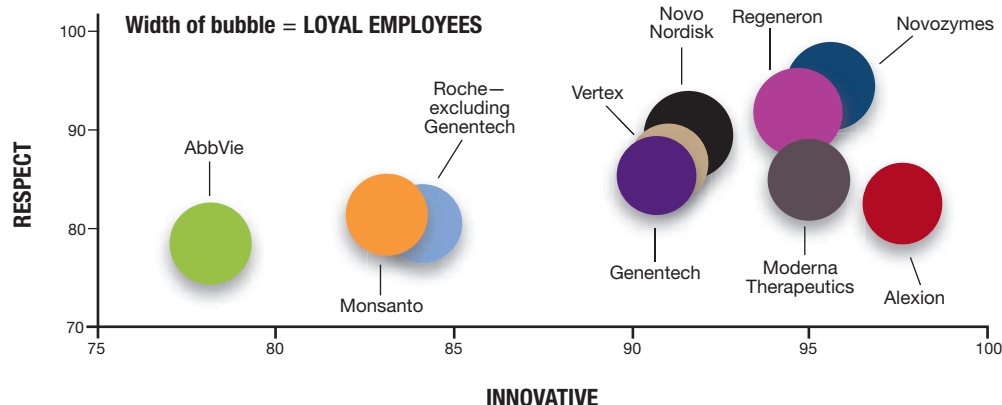
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Comparison of top ten's top characteristics



Comparison of the top 10 companies on the basis of the top three drivers (scored out of 100):

- **Innovative leader in the industry** (x-axis),
- **Treats employees with respect** (y-axis),
- **Has loyal employees** (bubble width).

show the capacity for managing uncertainty, change, and even ambiguity in a fast-moving company, says Moderna's **Steve Harbin**, senior vice president for human resources. "I look for it in every interviewee, because the one constant is that Moderna is changing," he says.

When hiring at Roche, Pao says that personality is equally as important as a deep understanding of disease biology and an appreciation of drug development. "We look for a team player who can fit into a matrix environment," he says, echoing other top employers as well. That means someone who can ferry his ideas and data between all the various layers of a drug development program—from target discovery to chemistry to preclinical tools and testing, clinical development, and beyond.

Respect begets loyalty

It's an old maxim of business: People leave employers because of bad managers. This year, the second-most important driver of top employers was respecting employees, followed closely by having loyal employees. Workers at the best companies say that respect, which can take many forms, begets loyalty. At Novozymes, employee turnover is low—just 8.5% worldwide in 2014—reflecting a very low proportion of heavy-handed managers, says **Michael Almer**, vice president of human resources.

The company also puts a Scandinavian twist on trust—giving employees a hefty dose of responsibility upfront. Cude recalls being handed that mantle on day one, starting in an empty lab with 12 others at the new agbiotech facility. "We still had these goals and timelines dictated by the growing season to meet," he says. "I was put into the deep end, but it was a really great learning and networking experience."

Smaller tokens of employee appreciation don't hurt either. Companies have brought in "jeans every day" dress codes (Alexion), free ice cream trucks (Novozymes), and an electric car for zipping between campuses (Moderna).

More serious benefits make high-performance employees' lives a little less stressful. Alexion provides paid caregiver leave to spend time with a terminally ill loved one and coaches for families navigating college applications. Celgene places a hefty emphasis on employee wellness, employing a nurse practitioner to treat employees on-site and providing hot, healthy to-go dinners from its cafeteria and local, farm-fresh produce for employees to buy on their way home. Moderna operates on the cantina model, serving a free catered lunch so employees can discuss matters over the daily meal.

But Harbin pooh-poohs the idea that perks like foosball tables will reel in or retain employees. "So Moderna provides free lunch, who cares?" he dismisses. Providing an environment where employees can dream up new ideas and carry them out is more important. "It is the speed with which we move from ideation to execution that makes Moderna special."

Unbridled enthusiasm

That buzz for getting things done permeates all top employer companies and shines through in this year's historically positive overall survey scores. Even though the global economic outlook has ticked upward, industry leaders attribute the survey's optimistic attitudes to scientific excitement rather than financial security.

"It's unique to be at a company sitting on top of discoveries that are actually changing the standard of care in cancer," says **Carl Decicco**, head of discovery at BMS. Four of the company's immuno-oncology drug trials had to be stopped due to the ethical need to offer the more effective experimental treatment to the other arm of patients receiving standard care. "BMS is willing to take risks that are backed by good scientific data," he says. "We're getting a lot of things right and people are finding it an exciting place to work."

Not resting on laurels inspires Celgene's employees to always strive for the next level of success, says Chief Financial Officer **Peter Kellogg**. "We try to keep the money focused on the science and innovation." He says employees appreciate long-range planning and vision that allows companies to ride out shifting financial trade winds. "There's something to be said for persistence and never feeling like we are a successful company."

Above all else, employees rank innovations that allow them to make a positive, real impact in the world—not compensation, retirement benefits, or career advancement—as the biggest reward of working in biotech and pharmaceuticals. Blasiak refers to an oft-repeated motto at Novozymes about having a "triple" bottom line.

"'People, planet, profit'—which is totally buzz-wordy—but it really does mean something here," she says. Because the company's leaders truly care that profitable products also do some good for people and the environment, Blasiak and her colleagues are inspired to put forward their best effort. "At the end of the day, everyone wants their work to be doing some good."

Kendall Powell is a freelance science writer based in Lafayette, Colorado.
DOI: 10.1126/science.opms.r1500160



CHINESE ACADEMY OF SCIENCES JOHN INNES CENTRE

Centre of Excellence for Plant and Microbial Sciences (CEPAMS)

CEPAMS Group Leader Positions

The Chinese Academy of Sciences (CAS) Institute of Genetics and Developmental Biology (Beijing) and the CAS Shanghai Institute of Plant Physiology and Ecology, together with the John Innes Centre in the UK, have established a joint Centre of Excellence for Plant and Microbial Sciences (CEPAMS).

We wish to recruit up to ten new tenure track Group Leaders to join this Centre, to be based either in Beijing or Shanghai. Group Leaders will run internationally outstanding and innovative research programmes. We are particularly interested in candidates in the areas of plant developmental genetics, crop improvement and plant and microbial natural products, but invite candidates from any field of plant and microbial sciences to apply.

These positions will be affiliated with both the Chinese Academy of Sciences and the John Innes Centre. Successful candidates will be employed by CAS and based in new and dedicated CAS laboratories in either Beijing or Shanghai. Successful candidates will be expected to also apply to the CAS Pioneer Hundred Talents Program or the National Thousand Young Talents Program.

Application forms and further information can be obtained from **Christopher DARBY** at **christopher.darby@jic.ac.uk (+44 1603 450970)**. Applications should be submitted to **Lei QI** by e-mail to **lqi@genetics.ac.cn (+86 10 64806505)**.

Applications will be welcomed until all ten positions are filled. There is no closing date, but the first sift for potential applicants will take place from **14 December 2015**. The first round of interviews will take place in Shanghai between 15 and 18 March 2016.

This recruitment exercise and any subsequent appointment are exclusively regulated by the employment law of the People's Republic of China.



Institute of Genetics and Developmental Biology

Chinese Academy of Sciences

Faculty Positions

The Institute of Genetics and Developmental Biology (IGDB), Chinese Academy of Sciences is a leading life science institution in China, and aims to address fundamental questions in modern agriculture and human health, making internationally recognized contributions in these fields.

IGDB invites applicants for ten faculty positions. The appointments will be at Full, Associate or Assistant Professor Level. We are particularly interested in candidates in the areas of molecular genetics, genomics, crop design, genome engineering, stem cells and regenerative medicine, organ development, metabolic health, computational biology and systems biology. In addition, positions are also open for biotech IP management and product development.

Successful candidates will be expected to also apply to the CAS Pioneer Hundred Talents Program or the National Thousand Talents Program. Applications will be welcomed until all positions are filled.

More information about IGDB can be found at **<http://www.genetics.ac.cn>** or **<http://english.genetics.cas.cn>**. Interested candidates should submit a cover letter, curriculum vitae, representative publications, a statement of research experience and interests as well as the names and contact information of three referees to **Dr. Weicai Yang** by e-mail (**wcyang@genetics.ac.cn**), and **Ms. Jing Wang** (**jwang@genetics.ac.cn**).

Yale Cancer Biology Institute

The Cancer Biology Institute, part of the Yale Comprehensive Cancer Center (YCC) at Yale University School of Medicine, invites applications from basic and physician scientists with expertise in computational biology for an Assistant, Associate, or Full Professor position in the tenure track. Appointment at Yale School of Medicine is available in a number of departments, and rank will be commensurate with experience. Applicants must have an M.D. and/or Ph.D. or equivalent degree and have demonstrated excellent qualifications in research and education. The successful candidate will have a strong background in high-end computational approaches as applied to theory, simulation, and/or statistical approaches to questions in biology at any scale, as well as a strong record of applying these approaches to important problems in cancer biology. Responsibilities include establishing a vigorous and independently funded research program in computational cancer biology while supervising and mentoring students with diverse backgrounds, as well as contributing to the graduate and medical school educational missions. We seek individuals with strong records of independent creative accomplishments, who will interact productively with colleagues within the Cancer Biology Institute, across the Yale West Campus, and across the Yale Comprehensive Cancer Center – taking advantage of the unique opportunities to translate basic findings from the Institute into clinical practice.

The Cancer Biology Institute is one of 7 newly-formed multi-disciplinary research institutes on Yale's burgeoning West Campus, which is self-contained but conveniently linked to Yale's nearby New Haven campuses. Additional West Campus assets include the Systems Biology Institute, the Nanobiology Institute, the Chemical Biology Institute, and the Microbial Sciences Institute – as well as state-of-the-art core facilities in imaging, small molecule discovery, genomic analysis and other technologies. Linkage to these Institutes provides unique opportunities for interdisciplinary research in the Cancer Biology Institute that is simultaneously poised for translation through close involvement with the YCC and Smilow Cancer Hospital at Yale-New Haven.

Please submit a letter describing qualifications, with a CV and three letters of reference to:

Drs. Mark Lemmon and Joseph Schlessinger
Co-Directors, Cancer Biology
Institute, Yale Cancer Center
 c/o Nathan Kucera
 333 Cedar Street
 PO Box 208066
 New Haven
 CT 06520-8028
 or send electronically to
cancerbiologyinstitute@yale.edu

We seek candidates who embrace and reflect diversity in the broadest sense. Yale University is an Equal Opportunity/Affirmative Action Employer. Review of applications will begin immediately and will continue until the position is filled.

Gan & Lee Pharmaceuticals Is Seeking Talents



Gan & Lee Pharmaceuticals (www.ganlee.com) is an innovative biotech company based in the southern suburb of Beijing, China. The company is experiencing rapid growth. We plan to significantly increase our research and development capability in the coming years, including establishing the Gan & Lee Laboratories and a Clinic Development Center in the US. We are recruiting talented, productive and ambitious scientists and professionals to join our highly motivated research team.

Scientists at various levels (Job code: SCI033) The candidates must have a Ph.D. degree in biology or chemistry. Postdoctoral training in relevant fields is strongly recommended. We expect the candidates to have demonstrated independent research capability and excellent productivity with strong publication record. Drug-development experience is NOT required.

The successful candidates will be compensated competitively according to their qualifications. The compensation package includes a salary starting from \$65,000 for entry-level positions and \$150,000 for the senior position (from **scientists to principle scientist**), plus bonus and generous stock option plans. The Beijing-based positions will be compensated with equivalent RMB. We have immediate openings of five entry-level positions and one senior position. But we will never turn down a talented scientist.

The scientists will work in Beijing- and/or US-based R&D laboratories, which are joined by many independent and collaborating labs, not dissimilar to an integrated graduate program in academics. Each lab, led by a principle investigator (you could be one of them), carries on one or more drug development programs, initiated by the PIs or assigned by the management. In addition, the PIs are allowed to spend up to 20% of time and effort to engage in their own research interest. Publication is highly encouraged.

Managing Director, Medical/Clinical Operations (Job code: MED009) This is a full time position. The incumbent reports to board of directors of Gan & Lee USA and leads the clinical development of the diabetes disease area. The successful candidate will function as a scientific and medical resource for the Medical Affairs department at Gan & Lee Pharmaceuticals, and as a medical monitor for ongoing clinical trials. For detailed responsibilities and qualifications, please visit:

<http://www.ganlee.com/en/index.php?optionid=685>

Manager, Regulatory Affairs (Job code: REG012) This is a full time position. Working closely with Associate Director of Regulatory Affairs, the incumbent will be responsible for outlining the regulatory strategy, coordinating activities related to regulatory interactions, such as global regulatory filing, meetings, etc. For detailed responsibilities and qualifications, please visit:

<http://www.ganlee.com/en/index.php?optionid=685>

Interested candidates can submit their applications electronically to hrrd@ganlee.com. Please indicate the job code in the subject field. In addition to your CV, please also include a one-page essay briefly highlighting your accomplishments, research interests and career goal.



Associate or Full Professor Biochemistry and Cell Biology

The Department of Biochemistry and Cell Biology at Stony Brook University invites applications for a faculty position at the tenured Associate or Full Professor level. Candidates doing innovative work in any area of biochemistry, molecular or cell biology are encouraged to apply. We particularly welcome applications from candidates working at the interface of Cancer and Metabolism.

Candidates must have an MD and/or Ph.D. and a strong record of significant scholarship and extramural funding. The opportunity also exists for the successful applicant to play a leadership role in the newly formed Biomedical Sciences Institute. The Institute will house multi-departmental collaborative teams of investigators and serve as the administrative hub for the Basic Science departments of Stony Brook Medical School.

Comprised of faculty in both the College of Arts and Sciences, and the School of Medicine, the Department of Biochemistry and Cell Biology is an active, collaborative community of scholars with broad research interests dedicated to both scientific discovery and to education at the undergraduate, graduate and post-graduate levels. Beyond the Department there are opportunities for collaboration with other research programs including the Cancer Center, the Laufer Center for Physical and Quantitative Biology, and the Institute for Chemical Biology and Drug Discovery. In addition, the University maintains professionally staffed core facilities to support research including ones for proteomics, next generation sequencing, NMR spectroscopy, and microscopy.

Interested individuals should complete the Academic Jobs application process online at <https://academicjobsonline.org/ajo/jobs/6464>. The application process consists of: 1) cover letter, 2) curriculum vitae, 3) teaching statement, 4) research statement, 5) State employment application, 6) three reference letters 7) publication list. Electronic submission via Academic Jobs Online is highly preferred.

Alternatively, submit above mentioned materials to: **Chair of the Search Committee, c/o Carol Juliano, Department of Biochemistry and Cell Biology, Life Sciences Building, Room 450, Stony Brook University, Stony Brook, NY 11794-5215, Fax: (631) 632-8575.**

For a full position description, or to apply online visit,
www.stonybrook.edu/jobs (Ref. # F-9566-15-10).

Equal Opportunity Employer, females, minorities, disabled, veterans.



Biological
Discovery
in Woods Hole

The Marine Biological Laboratory 2016 Whitman Center Fellowship Program



THE MARINE BIOLOGICAL LABORATORY, a hub for research and education and an affiliate of the University of Chicago, convenes biologists from around the world each year to advance the mission of biological discovery. We are now accepting applications for Whitman Center Fellowships for the 2016 season. Support is available for scientists to come to the Marine Biological Laboratory for 4 to 10 weeks to conduct independent research.

We particularly encourage applications focused on the following:

- Evolutionary, genetic, and genomic approaches in regenerative and developmental biology and neuroscience with an emphasis on novel marine organisms
- Integrated imaging and computational approaches to illuminate cellular function and biology emerging from the study of marine and other organisms

The 2016 Fellowship Program includes **Whitman Early Career Investigator Awards**, specifically designated for individuals less than 10 years from their doctoral degree who wish to focus on these areas of biological discovery.

Whitman Center Fellowships cover laboratory rental and housing costs. The Whitman Center offers access to state-of-the-art instrumentation, innovative imaging technology, genome sequencing, availability of model freshwater and marine organisms, and modern laboratory facilities.

Whitman Fellows typically come to Woods Hole during the summer months when the Marine Biological Laboratory hosts hundreds of researchers, postdocs, and graduate students from around the world to participate in scientific discovery courses, research, lectures, and field studies. As a convener of biology, the Marine Biological Laboratory is well known for fostering a highly collaborative environment, with scientists and students engaged in intensive research in a collegial and informal atmosphere.

Applications will be evaluated on the basis of scientific merit. Eligible applicants must hold appointments at accredited universities, colleges, or research institutions anywhere in the world. Researchers who have received Whitman Center Fellowships more than twice previously are not eligible. The Marine Biological Laboratory is especially interested in qualified candidates who can contribute to the diversity and excellence of its research community.

mbl.edu/research/whitman-fellowship
researchprograms@mbl.edu

Application Deadline: **December 15, 2015**



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clusterhiring.ucr.edu

Yale Cancer Biology Institute

The Cancer Biology Institute, part of the Yale Comprehensive Cancer Center (YCC) at Yale University School of Medicine, invites applications from basic and physician scientists for an Assistant, Associate, or Full Professor position in the tenure track. Appointment at Yale School of Medicine is available in a number of departments, and rank will be commensurate with experience. Applicants must have an M.D. and/or Ph.D. or equivalent degree and have demonstrated excellent qualifications in research and education. The successful candidate will have experience in the field of cancer biology, with specific target areas for this recruitment being cancer proteomics, metabolomics/metabolism, bioinformatics, cancer genetics and genomics, chemical biology, RNA/transcriptomics, tumor microenvironment, and tumor immunology. Responsibilities include establishing a vigorous and independently funded research program in cancer biology while supervising and mentoring students with diverse backgrounds, as well as contributing to the graduate and medical school educational missions. We seek individuals with strong records of independent creative accomplishments, who will interact productively with colleagues within the Cancer Biology Institute, across the Yale West Campus, and across the Yale Comprehensive Cancer Center – taking advantage of the unique opportunities to translate basic findings from the Institute into clinical practice.

The Cancer Biology Institute is one of 7 newly-formed multi-disciplinary research institutes on Yale's burgeoning West Campus, which is self-contained but conveniently linked to Yale's nearby New Haven campuses. Additional West Campus assets include the Systems Biology Institute, the Nanobiology Institute, the Chemical Biology Institute, and the Microbial Sciences Institute – as well as state-of-the-art core facilities in imaging, small molecule discovery, genomic analysis and other technologies. Linkage to these Institutes provides unique opportunities for interdisciplinary research in the Cancer Biology Institute that is simultaneously poised for translation through close involvement with the YCC and Smilow Cancer Hospital at Yale-New Haven.

Please submit a letter describing qualifications, with a CV and three letters of reference to:

Drs. Mark Lemmon and Joseph Schlessinger
Co-Directors, Cancer Biology Institute
Yale Cancer Center
c/o Nathan Kucera
333 Cedar Street
PO Box 208066
New Haven
CT 06520-8028

or send electronically to
cancerbiologyinstitute@yale.edu

We seek candidates who embrace and reflect diversity in the broadest sense. Yale University is an Equal Opportunity/Affirmative Action Employer. Review of applications will begin immediately and will continue until the position is filled.



Department of Health and Human Services National Institutes of Health National Institute on Aging Diversity in Aging Research Pipeline Program Post-doctoral Positions



The National Institute on Aging (NIA), a research component of the National Institutes of Health (NIH) and the Department of Health and Human Services (DHHS) is the lead federal agency for aging, age-related disease, and Alzheimer's disease research. The NIA Intramural Research Program through its **Diversity in Aging Research Pipeline Program (DARPP)** is advertising two postdoctoral fellowship positions. The goal of **DARPP** is to enhance diversity within the workforce of aging researchers and to provide training opportunities in aging research for underrepresented minorities and students from socioeconomically disadvantaged backgrounds. Successful candidates will be exceptionally qualified scientists-in-training who are interested in joining the NIA research community. Candidates must have a Ph.D. (or M.D.) degree in molecular biology, biochemistry, bio-informatics, genomics, epidemiology, neuroscience or a related biomedical science field. All applicants must be from a population underrepresented in the biomedical sciences, and have less than 5 years of postdoctoral experience. Candidates may be U.S. citizens or permanent-residents. Salary will be commensurate with research experience, according to the NIH intramural pay scale. Interested applicants should e-mail curriculum vitae, a brief description of their research interests, and 3 letters of reference to: The DARPP Selection Committee c/o Mrs. Taya Dunn Johnson, E-mail: dunnt@grc.nia.nih.gov. The deadline for application is **February 1, 2016**. For more information about the NIA intramural program, please visit <http://www.grc.nia.nih.gov/>

HHS and NIH are Equal Opportunity Employers

The NIH is dedicated to building a diverse community in its training and employment programs.



Faculty Positions Department of Pharmacology

The Department of Pharmacology at the University of Michigan Medical School invites applications for a tenured/tenure-track position at the **ASSISTANT, ASSOCIATE or FULL PROFESSOR** level. We are seeking outstanding individuals with research experience and interests that augment current department initiatives in the area of **ion channel research**. Qualifications include a Ph.D. in Pharmacology, or a related discipline, and/or a M.D. degree, and for those applying above the level of Assistant Professor, a strong record of nationally competitive external funding, a sustained record of excellent research productivity, and an outstanding national reputation in their field of interest. Physician-Scientists are encouraged to apply, as joint appointments are available with clinical departments within the University of Michigan Medical School. Applicants will be expected to maintain extramural funding, participate in the teaching of medical, graduate, and undergraduate courses, and to support and mentor graduate students and postdoctoral fellows. An attractive startup package including excellent laboratory space and generous funding is available. Salary will be commensurate with experience.

The successful candidates will join a dynamic, diverse, and collaborative department with new leadership in a Top 10 Medical School in a university setting with superb opportunities for continuing career development. The quality of life in Ann Arbor is outstanding. The combination of a large, major research university with a diverse, safe, family-oriented community make Ann Arbor an ideal environment for work-life balance. Ann Arbor offers an exceptional combination of sports, recreation, and cultural events.

Applicants should send a cover letter stating the position and subject area for which they are applying and the names and contact information of three referees, their *curriculum vitae*, a three-page summary of their research program and future research plans, and information related to past and current teaching experience as a single PDF file to jdani@umich.edu. Address all correspondence to: **Dr. Leslie Satin, Chair, Search Committee, Department of Pharmacology, The University of Michigan Medical School, 1150 West Medical Center Dr., Ann Arbor, MI 48109-5632.**

Review of applications will begin on **November 1, 2015**, and will continue until the position is filled.

The University of Michigan is an Affirmative Action/Equal Opportunity Employer. Applications from qualified women, minorities and/or disabled individuals are encouraged.

Launched in 2014, the Evergrande Center for Immunologic Diseases at Harvard Medical School and Brigham and Women's Hospital comprises both basic and translational research programs focused on chronic inflammation underlying autoimmune, neurologic and metabolic diseases, and the environmental factors that trigger chronic inflammation. The Center has an ambitious ten-year growth plan, including faculty recruitment.

Evergrande Center faculty:

- Vijay Kuchroo (Chair)
- Arlene Sharpe (Co-Chair)
- Ana Anderson
- Christophe Benoist
- Diane Mathis
- Howard Weiner



We are an equal opportunity employer and all qualified applicants will receive consideration for employment without regard to race, color, religion, sex, national origin, disability status, protected veteran status, or any other characteristic protected by law.

EVERGRANDE Center for Immunologic Diseases



Assistant Professor

The Evergrande Center is inviting applications for a tenure track position at the rank of Assistant Professor commensurate with experience and accomplishments. We seek an exceptional scientist addressing fundamental or translational questions related to chronic inflammation.

Applicants should have a track record of high-quality, published research, and proposals for an exciting line of investigation related to chronic inflammation. The successful candidate will join the current six core faculty members of the Evergrande Center for Immunologic Diseases, where s/he will direct a program of independent research, taking advantage of the Center's core facilities, expertise and other resources. We are particularly interested in candidates who value a highly collaborative and interactive approach to research. The ideal candidate will have an MD and/or PhD and evidence of a creative, collegial and exceptional research track record. While a track record of grant funding is desirable, it is not required.

The appointment will be with the Department of Neurology at Brigham and Women's Hospital and the Department of Microbiology and Immunology at Harvard Medical School. The successful candidate will be provided laboratory space in a new research building due to open 2016, and a customary start-up package. Salary and benefits will be competitive with other institutions.

Interested candidates should send a cover letter, a curriculum vitae, and a brief (2-3 pages) statement of current and future research plans to the Chairs of the Committee (Vijay Kuchroo and Arlene Sharpe) at:

Admin@evergrande.hms.harvard.edu

Computational Biologist

The Computational Biologist will coordinate and perform computational analyses of the genomic and transcriptomic data generated within the Center's laboratories, including working with Center faculty to formulate strategies, assessing and applying software tools and programming languages, developing novel statistical tools and algorithms, orchestrating data integration and advising and training Center researchers. Additionally, the Computational Biologist will coordinate and lead input from other Center computational specialists and coordinate with Brigham and Women's Hospital and Harvard Medical School IT staff for access to high-performance computer resources and for the deployment and management of data analysis pipelines, databases and web servers. Special projects as assigned.

To apply for the Computational Biologist position, please visit the Brigham & Women's Hospital Career site

at: **careers.brighamandwomens.org**

and search for **Job ID # 3001286**.

For further details on these positions and the Evergrande Center, please visit our website at:

evergrande.hms.harvard.edu



The Department of Biochemistry at Duke University School of Medicine (www.biochem.duke.edu) is continuing its expansion and invites applications for several tenure-track positions at the Assistant Professor level and tenured positions at the Associate Professor level. We welcome candidates in all areas of biochemistry whose research focuses on elucidating the fundamental, molecular-level mechanisms that underlie biological processes. Successful candidates will be expected to establish a strong, independent research program and to participate in departmental teaching and service.

Electronic applications should be sent as a single pdf file that includes a cover letter, curriculum vitae, and an approximately three-page summary of research experience and future plans. The application should be sent to **facultysearch-biochem@win.duke.edu**. Letters of recommendation should be sent directly by three referees as pdf files to **rec-biochem@win.duke.edu**. To ensure full consideration, applications should be completed by **December 1, 2015**.

Duke University is an Affirmative Action/Equal Opportunity Employer committed to providing employment opportunity without regard to an individual's age, color, disability, genetic information, gender, gender identity, national origin, race, religion, sexual orientation, or veteran status.

Faculty Position in Immunology Department of Biological Sciences Purdue University

The Department of Biological Sciences seeks candidates for an academic year, tenure-track position in the area of Immunology at the level of Assistant Professor. Creative investigators employing cutting-edge approaches to study aspects of cellular and molecular immunology are encouraged to apply. Areas of interest include, but are not limited to, host-pathogen interactions, inflammation, immune responses to infectious diseases, and cancer/tumor immunology. This position is aligned with a major investment in the life sciences by the Office of the Executive Vice President for Research and Partnerships at Purdue University supporting the establishment of a Purdue Institute for Inflammation, Immunology and Infectious Disease (PI4D) (<https://www.purdue.edu/research/life-sciences/>).

Applicants must have a Ph.D. degree or equivalent in an appropriate discipline and at least 2 years of postdoctoral experience. The successful candidate is expected to maintain a dynamic and collaborative research program addressing fundamental questions in the areas listed above and to excel in teaching at the undergraduate and graduate levels. Opportunities for collaboration with microbiologists, structural biologists and cell biologists within the Department of Biological Sciences (<https://www.bio.purdue.edu/>) are further enhanced by interactions with faculty from the Colleges of Science, Pharmacy, Veterinary Medicine and Engineering and the NCI-designated Purdue University Center for Cancer Research (<http://www.cancerresearch.purdue.edu/>). Abundant infrastructural support for research is provided by core facilities in the Bindley Bioscience Center, the Birck Nanotechnology Center and other centers within Discovery Park (<http://www.purdue.edu/discoverypark/>). As an ADVANCE institution, Purdue University is dedicated to the recruitment, retention, and advancement of women STEM faculty.

Applications should be submitted electronically to **<http://hiring.science.purdue.edu>** as a single PDF containing a letter of interest, detailed curriculum vitae, contact information for three referees, a 2-3 page summary of research interests and a one page teaching statement. The Department of Biological Sciences is committed to advancing diversity in all areas of faculty effort, including scholarship, instruction, and engagement. Candidates should address at least one of these areas in their cover letter, indicating their past experiences, current interests or activities, and/or future goals to promote a climate that values diversity and inclusion. Inquiries should be directed to **Elizabeth J. Taparowsky, Chair, Immunology Search Committee, at ImmunoSearch@bio.purdue.edu or at Department of Biological Sciences, 915 State Street, West Lafayette, IN 47907-2054**. A background check is required for employment in this position. A review of applications will begin on **December 1, 2015**, and continue until the position is filled.

Purdue University is an EOE/AA Employer. All qualified applicants, including minorities, women, and individuals with disabilities and veterans are encouraged to apply.



THE UNIVERSITY OF HONG KONG



Research Officer in the Department of Chemistry (Ref.: 201501233)

Applications are invited for appointment as Research Officer in the Department of Chemistry, to commence as soon as possible, on a three-year fixed-term basis, with the possibility of renewal subject to satisfactory performance.

Applicants should possess a Ph.D. degree with extensive experience in X-ray crystallography in academic institutes/industrial sector. They should have in-depth knowledge of and extensive experience in the practical aspects of small-molecule and powder crystallography, including crystallization, data acquisition, structure solution and refinement. The appointee will be responsible for providing structure determination service while supporting the data collections and structure determinations by students, and the daily operation and regular maintenance of X-ray facilities in the Department. He/She will also provide assistance for the development of crystallographic research methodology, and training to research students and authorized users for data collection and analysis.

A globally competitive remuneration package commensurate with qualifications and experience will be offered. At current rates, salaries tax does not exceed 15% of gross income. The appointments will attract a contract-end gratuity and University contribution to a retirement benefits scheme, totalling up to 15% of basic salary, as well as annual leave, and medical benefits. Housing benefits will also be provided as applicable. Please note that the University is not able to offer a relocation assistance package (including housing accommodation and a passage and baggage allowance) to the successful candidate recruited from overseas.

Applicants should send a completed application form together with an up-to-date C.V. by e-mail to chemjob@hku.hk. Application forms (341/1111) can be downloaded at <http://www.hku.hk/apptunit/form-ext.doc>. Further particulars can be obtained at <http://jobs.hku.hk/>. **Closes November 30, 2015.**

The University thanks applicants for their interest, but advises that only candidates shortlisted for interviews will be notified of the application result.

The University is an equal opportunities employer and is committed to a No-Smoking Policy

VANDERBILT UNIVERSITY

MEDICAL CENTER

FACULTY POSITION IN MICROBIAL PATHOGENESIS

The Department of Pathology, Microbiology and Immunology at Vanderbilt University Medical Center invites applications for a tenure-track faculty position at the Assistant Professor level (PhD, MD, MD/PhD). Areas of particular interest include, but are not limited to, viral pathogenesis, bacterial pathogenesis, and host-microbe interactions. Successful candidates will be expected to establish and maintain an independent research program and participate in teaching of graduate and medical students. Candidates should have substantial post-graduate training highlighted by peer-reviewed publications that demonstrate research productivity.

Vanderbilt University Medical Center, located on the Vanderbilt University campus, is home to internationally recognized programs in bioinformatics, drug discovery, global health, inflammation, imaging science, pharmacology, proteomics, and vaccine science. The School consistently ranks in the Top 20 US Medical Schools and provides outstanding opportunities for scholarship, collaboration, and teaching.

The Vanderbilt University campus is a National Arboretum located in the heart of Nashville, the capital of Tennessee, known internationally as "Music City USA". Nashville is also the home to professional sports teams, the Nashville Symphony, the Frist Center for the Visual Arts, and numerous activities for outdoor enthusiasts. Nashville, Tennessee is a wonderful place to live, work, and raise a family.

Applicants should send a *curriculum vitae* and a statement of current and future research interests, and at least 3 references to: **Eric Skaar, Ph.D.**, Vice Chair for Basic Research and Director, Division of Molecular Pathogenesis, Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center at his Email: micropath@vanderbilt.edu

Vanderbilt University is an Affirmative Action/Equal Opportunity Employer. Women and minority candidates are encouraged to apply.



Georgia Institute of Technology

Chair of the School of Biology

The Georgia Institute of Technology invites applications for the Chair of the School of Biology. We are a dynamic department with diverse research activities in areas including genomics, molecular biology, chemical biology, bioinformatics, ecology and evolution, and integrative approaches to complex biosystems. Our research programs have been highly successful in capitalizing on the engineering and computing expertise and access to world-class facilities at Georgia Tech. The School is strongly supported by campus-wide initiatives in the life sciences, including the Petit Institute for Bioengineering and Biosciences and the newly opened Engineered Biosystems Building. We have expanded with 14 new faculty hires during the last five years. The School seeks to continue its positive trajectory as a leader in interdisciplinary science and innovative educational practices. The School offers B.S., M.S., and Ph.D. degree programs in Biology, and participates in interdisciplinary degree programs in Bioinformatics and Quantitative Biosciences. Georgia Tech is situated on an attractive urban campus in the heart of Atlanta, a vibrant city with diverse cultural and economic opportunities.

We seek candidates with established excellence in leadership and scholarship, and who possess strong commitments to interdisciplinary research as well as undergraduate and graduate education. Candidates with a demonstrated interest in promoting diversity in the sciences are encouraged to apply. Applications should be sent by e-mail to: biochair@cos.gatech.edu, and submissions arriving before **December 1, 2015** are guaranteed full consideration; subsequent applications may be considered until the position is filled. Applications should include a letter of interest and a curriculum vitae that demonstrates the applicant's research, teaching and leadership experience. For further information, please refer to the Georgia Tech Web page at <http://www.biology.gatech.edu/>.

Georgia Tech is a unit of the University System of Georgia and an Affirmative Action/Equal Opportunity Employer and requires compliance with Immigration Control Reform Act of 1986.

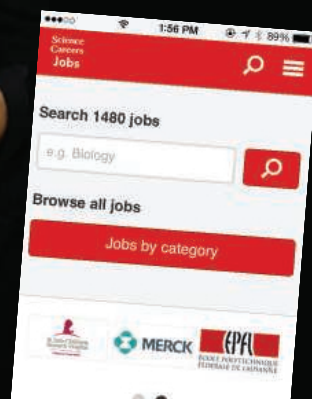
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暨南大學
JINAN UNIVERSITY

百年暨大，誠聘英才
攜手暨大，共創未來

Jinan University implements Jinan Double Hundred Talents Plan to recruit global talents

Jinan University, first university of overseas Chinese founded by the state, with the largest foreign student among the country currently, is the national "211 Project" of key comprehensive university directly under the guidance of Overseas Chinese Affairs Office of State Council. Jinan University, known as "Chinese top university".

According to the school discipline construction and innovative platform construction, Jinan University now recruiting global talents and will provide favorable working and living condition. There are multiple positions open at Economics College (applied economics, statistics, finance, regional economics) / Science and Technology College (Optical Engineering, Mechanical Engineering, Materials Science and Engineering, Physics, Food Science and Engineering) / Information Science and Technology College (Information & Communication Engineering, Electronic Science and Technology, Computer Science and Technology, Basic Mathematics, Computational Mathematics, Applied Mathematics, Cyberspace Security) / Life Science and Technology College (Chemical, Biological Engineering, Biochemistry and Molecular Biology, Cell Biology, Biology, Medical Engineering, Ecology, Developmental and Regenerative Biology, Immunobiology, Red Tide and Marine Biology)

Candidate material

- A resume
- Assumed research projects of nearly five years, published papers (specify the collection situation, journal impact factor and the number of papers he cited of SCI, EI, SSCI, CSCI), the list of award-winning achievements;
- Copy of Degree Certificates/Diploma, all research projects, awards and patents;
- The full text of 2-5 representative papers;
- Copy of certificate and incumbents certificate of holding an important position in the foreign office or in the domestic one;
- Work plan after coming to school.

Treatments provided

Jinan University provides 300,000-1,100,000 RMB annual salary for successful candidates, with 500,000-10,000,000 RMB research start-up fund and 1,000,000-5,000,000 RMB settling-in allowance.

Contact

Personnel department home page: <http://personal.jnu.edu.cn/>

Tel: 0086-20-85227283 (including fax), 0086-20-85223525

Contact: Mr. Tong, Mr. Liu

E-mail: otalents@jnu.edu.cn

Address: Human Resources Development and Management Service of Jinan University,

No. 601, Huangpu Road West, Guangzhou.

Postal Code: 510632

Job Vacancies in China's Universities



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Beijing University of Posts and Telecommunications(BUPT)

For more information about BUPT, please visit:
<http://rsc.bupt.edu.cn/>.

North China Electric Power University(NCEPU)

For more information about NCEPU, please visit:
<http://www.ncepu.edu.cn/rczp/index.htm>

Beijing Jiaotong University(NJTU)

For more information about NJTU, please visit:
http://jgrsc.bjtu.edu.cn/job/job_index.asp

Beijing Technology and Business University(BTBU)

For more information about BTBU, please visit:
<http://www.btbu.edu.cn/szdw/rczp/85256.htm>

For more details, visit <http://www.edu.cn/zhaopin>

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LABORATORY FOR LASER PLASMAS
SHANGHAI JIAO TONG UNIVERSITY

Multiple Faculty Positions and Postdoc Positions in Lasers, Plasma Physics, and Ultrafast Science

The Laboratory for Laser Plasmas at Shanghai Jiao Tong University, founded jointly by the University and the Ministry of Education of China in 2010, is a unique multidisciplinary research center. It brings together the best scientists, engineers, and educators from the fields of laser, plasma, accelerator physics and technologies, and ultrafast science to solve some key challenges of our modern society related to energy, environment, and health issues. The Laboratory has equipped with a series of state-of-the-art facilities including a multi-100 terawatt high power laser system, kHz terawatt laser systems, a high power mid-infrared laser system, a multi-MeV RF electron accelerator, advanced laser-target interaction chambers and diagnostics.

To further intensify our innovation capabilities, we are now seeking outstanding scholars to join us. The ideal candidates are supposed to have a PhD and a strong research record related to one of the following areas: high power laser technology, laser plasma theory and simulation, laser-plasma experiment, advanced particle accelerators and applications, high energy density physics, or ultrafast science. The following positions are available according to the candidates' experience:

(a) Distinguished professors selected by "National 1000 Talent Plan", "Changjiang Scholars Program", or "National Science Fund for Distinguished Young Scholars".

(b) Distinguished researchers selected by "National 1000 Young Talent Plan", "National Science Fund for Excellent Young Scholars".

(c) Postdoctors with a strong record of academic accomplishments (normally appointed for two years for this position).

For positions (a) and (b), the following qualifications are required at least: Working experience as a professor, associate professor or equivalent at top overseas universities or research institutes; Competence of leading a research team to carry out world-class scientific researches; Competence of cultivating young researchers and promoting international academic collaboration. For successful candidates, the University will provide attractive annual salary, start-up fund, housing allowance together with other benefits from the Laboratory. For positions (c), there is a good opportunity to be promoted to research staff provided excellent performance is achieved during the postdoc period.

All applicants should send a cover letter, a curriculum vitae with a publication list, a research proposal (3-4 pages), and a statement of teaching interest in a single pdf file by February 28, 2016 to Ms. Dan Zhang through e-mail: laser_plasma@sjtu.edu.cn. Please also arrange three reference letters directly to the above e-mail address. Applications after the deadline could be reviewed until the positions are filled.



Associate or Full Professor

The Department of Biology at Drexel University (www.drexel.edu/biology) is seeking applications for a senior scientist at the level of Associate or Full Professor. We seek an established scientist who has a national/international reputation, a well-funded, highly successful independent research program and an established record of scholarly and educational accomplishments. The successful applicant will use a variety of approaches to address fundamental questions in biology. We are particularly interested in individuals studying genomics, structure and function of the nucleus, and/or cell and organismal stress responses. This individual should enhance the strengths within the Department of Biology and promote strong interactions and collaborations with other departments throughout the various colleges of Drexel University. Competitive compensation and start-up packages are available. The anticipated start date for this position will be September 2016. Applicants should have a Ph.D., M.D., or D.Phil. as well as a significant record of external funding.

Please apply through Drexel Jobs by accessing this link: <https://www.drexeljobs.com/applicants/Central?quickFind=80269> Please attach your CV, two-page statement of research interests and goals, and a statement on teaching philosophy and experience. The deadline for receipt of applications is **January 31, 2016**. After that applications will be considered until the position is filled.

Please address all correspondence to **Professor John R. Bethea, Head of Biology and Chair of the Biology Search Committee, Dept. of Biology, Drexel University, 3245 Chestnut St., Philadelphia, PA 19104 (biology.search@drexel.edu)**.

Drexel University is an Equal Opportunity/Affirmative Action Employer and encourages applications from women, members of minority groups, disabled individuals, and veterans.



STATE UNIVERSITY OF NEW YORK
COLLEGE OF OPTOMETRY

FACULTY POSITION IN OCULAR PHYSIOLOGY

The Graduate Center for Vision Research at the State University of New York, College of Optometry invites applications for a tenure-track position from individuals with interests in molecular, biochemical, or cellular techniques to study the physiology of the eye. The successful candidate will have strong translational and clinical research interests that compliment existing research strengths. The successful candidate will be expected to maintain an externally funded research program and teach in the professional optometry degree (OD) and graduate programs (MS and PhD). The Graduate Center for Vision Research consists of a scientifically vibrant community addressing a variety of basic and applied topics in vision science and includes the Clinical Vision Research Center where clinical research is performed in collaboration with the College's University Eye Center. The College is situated in the center of Manhattan's scientific, medical and cultural activities. Competitive salary and start-up funds will be provided.

Applications will be considered at all ranks. The position will remain open until filled. Applicants should provide a CV, statement of research interests, reprints of up to three papers, and the names and contact information of three references, to Mr. Canh Tran (ctran@sunyopt.edu), SUNY Optometry, 33 West 42nd St, New York, NY 10036.

For more information about the College, including our Annual Safety Report, visit www.sunyopt.edu/careers.

The SUNY College of Optometry is an affirmative action, equal opportunity employer and does not discriminate on the basis of race, color, national origin, religion, creed, age, disability, sex, gender identity, sexual orientation, familial status, pregnancy, predisposing genetic characteristics, military status, domestic violence victim status, criminal conviction, or retaliation.

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FROM THE JOURNAL SCIENCE AAAS

EDITOR-IN-CHIEF

The Journal of Biological Chemistry

The American Society for Biochemistry and Molecular Biology welcomes nominations and applications for the position of **editor-in-chief of The Journal of Biological Chemistry**. The JBC publishes original research that makes novel and important contributions to the study of the molecular and cellular bases of biological processes. The next editor-in-chief should be a public-facing thought leader, a committed advocate for authors and readers, a leader who listens and delegates, and an active researcher of significant accomplishment.

Candidates should possess:

- broad, general knowledge of biological chemistry;
- strategic planning experience;
- a commitment to publishing the very best science;
- an appreciation for data-driven decision making;
- the ability and desire to recruit outstanding scientists to serve as contributors, associate editors and editorial board members;
- a willingness to provide sustained and consistent editorial direction;
- proven interpersonal, communication, leadership and coalition-building skills;
- financial and business prowess; and
- scientific editorial experience.

The editor-in-chief will:

- provide visionary strategic direction,
- act as the steward of the journal's scientific content;
- report results and next steps to ASBMB executives and elected leadership;
- establish and refine journal policies and editorial guidelines;
- lead inclusive, productive meetings for board members and associate editors;
- respond to media requests;
- collaborate with staff members and vendors;
- represent the journal at meetings and other venues; and
- write quarterly (or more frequent) editorials.



The editor-in-chief will serve a five-year term, with the possibility of reappointment. The ASBMB will provide administrative support and a stipend.

A search committee appointed by the president of the ASBMB will review nominations and applications. An application package should include a cover letter, a one-page vision for the journal and a CV (of no more than four pages) highlighting relevant experience and achievements.

Send nominations and applications by January 1, 2016, to the ASBMB Editor-in-Chief Search Committee, c/o ASBMB Senior Director of Publications and Content Development, Nancy Rodnan (nrodnan@asbmb.org).

UNIVERSITY OF MIAMI



Tenure-Track Assistant/Associate Professor

The **Department of Physics** at the **University of Miami** invites applications from highly qualified persons for a faculty position in Astrophysics. This appointment will be made at the Assistant or Associate Professor rank to begin Fall 2016. Targeted research topics include, but are not limited to, experimental or observational work that is synergistic with ongoing activities in the department: cosmic microwave background polarization, mm-wave spectroscopy, studies of solar wind charge exchange, diffuse x-ray emission, large scale structures, and detector development. Candidates must have a Ph.D. in physics or related discipline, a demonstrated record of research achievements, and a strong commitment to teaching and mentoring students at the undergraduate and graduate levels. The physics department is located within the University's attractive Coral Gables campus in the greater Miami area, and has a wide-ranging research expertise and established Ph.D. program.

Application materials, including curriculum vitae with list of publications and statement of research plans, should be sent electronically (as a single PDF) to astrosearch@physics.miami.edu or to **Astrophysics Search Committee Chair, Department of Physics, University of Miami, Knight Physics Building, Coral Gables, FL 33124**. Applicants should arrange for three letters of recommendation to be sent to the same address. Review of applications will begin on **December 1, 2015** and continue until the position is filled.

The University of Miami is an Equal Opportunity Employer — Females/Minorities/Protected Veterans/Individuals with Disabilities are encouraged to apply. Applicants and employees are protected from discrimination based on certain categories protected by Federal law.

UNIVERSITY OF MIAMI



Tenure-Track Assistant/Associate Professor

The **Department of Physics** at the **University of Miami** invites applications from highly qualified persons for a faculty position focusing on experimental Energy/Materials research. This appointment will be made at the Assistant or Associate Professor rank to begin Fall 2016. Targeted research topics include, but are not limited to, energy harvesting, storage, conversion, and optoelectronics, encompassing hard and/or soft matter. The successful candidate is expected to interact with both physics and chemistry faculty. Candidates must have a Ph.D. in physics or related discipline, a demonstrated record of research achievements, and a strong commitment to teaching and mentoring students at the undergraduate and graduate levels. The physics department is located within the University's attractive Coral Gables campus in the greater Miami area, and has a wide-ranging research expertise and established Ph.D. program.

Application materials, including curriculum vitae with list of publications and statement of research plans, should be sent electronically (as a single PDF) to energysearch@physics.miami.edu or to **Energy Search Committee Chair, Department of Physics, University of Miami, Knight Physics Building, Coral Gables, FL 33124**. Applicants should arrange for three letters of recommendation to be sent to the same address. Review of applications will begin on **November 23, 2015** and continue until the position is filled.

The University of Miami is an Equal Opportunity Employer — Females/Minorities/Protected Veterans/Individuals with Disabilities are encouraged to apply. Applicants and employees are protected from discrimination based on certain categories protected by Federal law.

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Cryo-Electron Microscopy

Full Professor

The Department of Biochemistry & Molecular Biology, together with the OHSU Center for Spatial Systems Biomedicine (OCSSB) seek an established scientist for a leadership role within the OHSU/FEI Living Laboratory. The position is a tenure-track faculty position with primary responsibilities for research, graduate training and opportunities in medical education.



As part of the Knight Cancer Center, OCSSB is investing heavily in development of biomolecular imaging including multiple forms of electron and super-resolution microscopy. The recruit will provide leadership in the highest resolution activities, with additional recruitment and expansion of instrumentation (currently including a Titan Krios™), taking advantage of collaborations with FEI Inc. and Intel Corp. in Portland to develop new capabilities in image collection and processing.

Candidates should have extensive records of extramurally funded research in structural biology, interest in advancing the technology, leadership experience, and the vision to help EM realize its full potential in cancer and basic biomedical research. Applications will be considered from **December 1, 2015** until the position is filled. Please apply online at **www.OHSUjobs.com**, ref. **IRC49876**. Include a CV, contact information for three referees and a statement of research interests and plans. Enquiries may be directed to **Michael Chapman (chapmami@ohsu.edu)**.

OHSU is an Equal Opportunity, Affirmative Action Institution that encourages diverse applicants to consider this opportunity. Applicants with disabilities can request reasonable accommodation by contacting the Affirmative Action and Equal Opportunity department at 503-494-5148.

MICHIGAN STATE UNIVERSITY

Department of Microbiology and Molecular Genetics Tenure Track Faculty Position in Immunology

The Department of Microbiology and Molecular Genetics (MMG) at Michigan State University (<http://www.mmg.msu.edu>) seeks candidates at the Assistant, Associate, or Full Professor level in immunology. Of greatest interest are research programs focusing on immunity at the mucosal surface and/or interactions of pathogens or the microbiome with the immune system, although outstanding investigators in other areas of immunology will be considered.

The MMG Department is highly collegial and collaborative, with diverse research interests in molecular and cellular immunology, bacterial and viral pathogenesis, prokaryotic and eukaryotic genetics, systems biology, and molecular evolution. State-of-the-art research infrastructure is readily available to MMG investigators. The successful applicant is expected to develop (or maintain) a rigorous, externally-funded research program with national visibility. Teaching in graduate, professional, or undergraduate programs is expected.

Applicants should have a Ph.D., D.V.M., D.O., or M.D. or equivalent advanced degree(s). Applications must include: (i) a letter of application addressing the required position qualifications, (ii) a current c. v.; (iii) NIH Biosketch (including current and pending research support) in the new format (<http://grants.nih.gov/grants/guide/notice-files/NOT-OD-15-032.html>); (iv) a statement of not more than 2 pages describing future research and training plans, and including the impact the applicant seeks to have on the field over the next five years; (v) contact information for 3 references.

Submit application materials as a single PDF to: **<https://jobs.msu.edu>** (position #2250). Review of applications will begin immediately, and the position will remain open until filled. Questions regarding the position can be addressed to the chair of the search committee, **Dr. Kathryn Meek (kmeek@msu.edu)**.

MSU is an Affirmative Action, Equal Opportunity Employer committed to achieving excellence through workforce diversity in an inclusive culture. Applications and/or nominations of women, persons of color, veterans and persons with disabilities are encouraged. MSU is committed to supporting employees' work and personal life, and offers employment assistance to the spouse or partner of faculty candidates.



The USC Michelson Center for Convergent Bioscience (<http://michelsonmedical.org/portfolio-item/gary-michelson-center-convergent-bioscience/>) is a joint initiative between the USC Dornsife College of Letters Arts and Sciences and the USC Viterbi School of Engineering. It focuses on the convergence of engineering and the biological sciences at various scales (from the quantum and atomic, to the molecular, cellular, and organ) with particular emphasis on the understanding of how the various parts of the human body interact and the subsequent engineering of new solutions for human health. This new paradigm promises to permanently reconfigure the way biomedical advances are made and ensure that those advances are rapidly translated into interventions. The USC Viterbi School of Engineering invites applications from outstanding candidates for tenure-track positions at any faculty rank in the relevant engineering disciplines, including Biomedical Engineering, Chemical Engineering and Materials Science, Electrical Engineering, and Mechanical Engineering. Faculty under this initiative may be part of The Bridge@USC and may be housed in the new USC Michelson Center, under construction.

This search is part of a cross-departmental hiring initiative involving multiple departments and multiple openings in the Viterbi School. The School is committed to increasing the diversity of its faculty and welcomes applications from women, underrepresented minorities, veterans, and persons with disabilities. We welcome synergies between departments and value demonstrated abilities of successful applicants to work across disciplines. Applications for faculty appointment in any department in the Viterbi School, with particular interest in the following areas:

Aerospace and Mechanical Engineering – computational engineering, biodynamical systems.

Biomedical Engineering – systems cellular-molecular bioengineering, neuroengineering, and medical imaging with emphasis on clinical translation, theranostics or multi-modal imaging.

Chemical Engineering and Materials Science – systems and synthetic biology, drug delivery, immunoengineering, biomolecular, biomaterials, viral, cellular, and computational approaches.

Electrical Engineering – bio-electronics, bio-nanotechnology, biophotonics, brain engineering, image formation and analysis for next-generation microscopy, neuromorphic systems.

We are also interested in exceptionally accomplished candidates who can be transformational in areas of the search across other departments in the Viterbi School. Outstanding senior applicants who have demonstrated academic excellence and leadership, and whose past activities document a commitment to issues involving the advancement of women in science and engineering may be also considered for the Lloyd Armstrong, Jr. Endowed Chair, which is supported by the Women in Science and Engineering Program endowment. We expect all candidates to have a strong commitment to research, doctoral student mentoring, and teaching at the undergraduate and graduate levels. All applicants must have earned a doctorate in the respective areas or closely related fields by the date of appointment.

To receive full consideration, candidates should apply on-line at the School's faculty openings website corresponding to where there is most interest in having a primary appointment, found at **http://viterbi.usc.edu/academics/faculty/faculty_positions.htm**. Application materials generally include a cover letter, curriculum vitae, statement of research and teaching interests, and contact information for suggested references. Applications should be submitted by **December 31, 2015** to receive full consideration. Interested persons are welcome to contact the department Chair.

The USC Viterbi School of Engineering is among the top engineering schools. It counts 180 full-time, tenure-track faculty members, and it is home to the Information Sciences Institute. The school is affiliated with the Alfred E. Mann Institute for Biomedical Engineering, the Institute for Creative Technologies and the USC Stevens Center for Innovation. In addition to its commitment to faculty diversity, the USC Viterbi School of Engineering is committed to enabling the success of dual career families and fosters a family-friendly environment.

USC is an Equal Opportunity Educator and Employer, proudly pluralistic and firmly committed to providing equal opportunity for outstanding persons of every race, gender, creed and background. The University particularly encourages women, members of underrepresented groups, veterans and individuals with disabilities to apply.

By Stephen T. Jackson

Going where the science matters

Not long ago, I was a tenured professor on a sabbatical at the University of Oxford in the United Kingdom. One winter morning, reflecting on why we scientists work as hard as we do, I identified three primary drivers: curiosity, ambition, and idealism. We chase interesting problems, wherever they lead. We're a competitive lot, with career advancement an important incentive. And we're compelled by a desire to benefit society.

Later that week, an old friend sent a photo taken in the Adirondack Mountains during my first summer's fieldwork as a Ph.D. student. Looking at my 24-year-old face, framed by long black hair and a dreadful orange sweater, I asked myself, "What was on that young fellow's mind?" Clearly, he wanted to satisfy his curiosity about how climate change affected ecological communities. He was idealistic, with notions of applying his training to the cause of conservation. In his naiveté, he viewed personal career advancement as unimportant, even contradictory to the greater good.

What would he think of my grayer self, sitting in my Oxford chambers some 30 years later? Though I hadn't answered all the questions that gripped him, he'd be pleased that I'd learned a lot about climate change and ecology. He'd probably be happy that I'd had a successful career—though I expect he'd ask whether I hadn't been co-opted by "the system." And he'd be wondering in exactly what ways I had contributed to conserving the natural world. My honest answer: "Not many, beyond haranguing students, giving public lectures, and writing academic papers."

Not long after these reflections, some colleagues suggested that I look into a job opening at a federal science agency to lead a program aimed at bridging the gap between the climate-change research and the natural-resource management communities. My knee-jerk reaction was to say no. I was comfortably tenured, enjoying the honors and perks of an established academic career. But tugging at my coat sleeve was my younger self, asking me why I shouldn't reinvest my knowledge and experience into benefiting the environment.

With no good answers for him, and a reawakening idealism, I applied for the job. It's now been 3 years since I assumed directorship of the Southwest Climate Science Center in Tucson, Arizona. I've worked harder than I ever did as a professor. I've had to acquire new skills, and I'm still strug-



*"What would he think
of my grayer self ...
some 30 years later?"*

I'm doing so in the center's rich environment of multiple academic disciplines and professional cultures, and with the view to solve compelling real-world problems.

Is my job perfect? Certainly not. As in any administrative job, some tasks are neither interesting nor enjoyable, and government administration has unique aggravations. But on the positive side, I no longer suffer the exhausting high-passion, low-stakes squabbles that can arise among university faculty members. And, most importantly, I enjoy the added satisfaction of knowing that my center is having a real impact on conservation. So, looking again at that old photo, I wonder whether the grin on my face came from knowing that, one day, I would inform and influence conservation in a direct and tangible way. ■

Stephen T. Jackson is director of the Department of the Interior Southwest Climate Science Center, U.S. Geological Survey, in Tucson, Arizona. He is also a professor emeritus of botany at the University of Wyoming in Laramie and an adjunct professor of geosciences at the University of Arizona in Tucson. Send your story to SciCareerEditor@aaas.org.